Saving the HONEYBEE

The mysterious ailment called colony collapse disorder has wiped out large numbers of the bees that pollinate a third of our crops. The causes turn out to be surprisingly complex, but solutions are emerging • By Diana Cox-Foster and Dennis vanEngelsdorp

Dave Hackenberg makes a living moving honeybees. Up and down the East Coast and often coast to coast, Hackenberg trucks his beehives from field to field to pollinate crops as diverse as Florida melons, Pennsylvania apples, Maine blueberries and California almonds.

As he has done for the past 42 years, in the fall of 2006 Hackenberg migrated with his family and his bees from their central Pennsylvania summer home to their winter locale in central Florida. The insects had just finished their pollination duties on blooming Pennsylvanian pumpkin fields and were now to catch the last of the Floridian Spanish needle nectar flow. When Hackenberg checked on his pollinators, the colonies were "boiling over" with bees, as he put it. But when he came back a month later, he was horrified. Many of the remaining colonies had lost large numbers of workers, and only the young workers and the queen remained and seemed healthy. More than half of the 3,000 hives were completely devoid of bees. But no dead bees were in sight. "It was like a ghost town," Hackenberg said when he called us seeking an explanation for the mysterious disappearance.

We and other researchers soon formed an interdisciplinary working team that by December 2006 had described the phenomenon and later named it colony collapse disorder, or CCD. Curiously, Hackenberg's colonies stopped dying the following spring, but by that time only 800 of his original 3,000 colonies had survived. As Hackenberg spoke to colleagues around the nation, it became apparent that he was not alone. And a survey our team conducted in the spring of 2007 revealed that a fourth of U.S. beekeepers had suffered similar losses and that more than 30 percent of all colonies had died. The next winter the die-off resumed and expanded, hitting 36 percent of U.S. beekeepers. Reports of large losses also surfaced from Australia, Brazil, Canada, China, Europe and other regions. More recent data are not available yet, but some beekeepers say they have seen their colonies collapse this winter, too.

The bee loss has raised alarms because one third of the world's agricultural production depends on the European honeybee, *Apis mellifera*—the kind universally adopted by beekeepers in Western countries. Large, monoculture farms require intense pollination activity for short periods of the year, a role that other pollinators such as wild bees and bats cannot fill. Only *A. mellifera* can deploy armies of pollinators at almost any time of the year, wherever the weather is mild enough and there are flowers to visit.

Our collaboration has ruled out many potential causes for CCD and found many possible contributing factors. But no single culprit has been identified. Bees suffering from CCD tend

KEY CONCEPTS

- Millions of beehives worldwide have emptied out as honeybees mysteriously disappear, putting at risk nearly 100 crops that require pollination.
- Research is pointing to a complex disease in which combinations of factors, including farming practices, make bees vulnerable to viruses.
- Taking extra care with hive hygiene seems to aid prevention. And research into antiviral drugs could lead to pharmaceutical solutions.

—The Editors

CHARLES KREBS

DAVID YELLEN

DAVE HACKENBERG was the first beekeeper to alert U.S. entomologists to the inexplicable disappearance of worker bees, a sign of what is now known as colony collapse disorder, in the fall of 2006. By the end of the winter, more than 60 percent of his 3,000-odd colonies were dead; nationwide the loss was 30 percent.

The bees were all sick, but each colony seemed to suffer from a different combination of diseases.

to be infested with multiple pathogens, including a newly discovered virus, but these infections seem secondary or opportunistic—much the way pneumonia kills a patient with AIDS. The picture now emerging is of a complex condition that can be triggered by different combinations of causes. There may be no easy remedy to CCD. It may require taking better care of the environment and making long-term changes to our beekeeping and agricultural practices.

Even before colony collapse, honeybees had suffered from a number of ailments that reduced their populations. The number of managed honeybee colonies in 2006 was about 2.4 million, less than half what it was in 1949. But beekeepers could not recall seeing such dramatic winter losses as occurred in 2007 and 2008. Although CCD probably will not cause honeybees to go extinct, it could push many beekeepers out of business. If beekeepers' skills and know-how become a rarity as a result, then even if CCD is eventually overcome, nearly 100 of our crops could be left without pollinators—and large-scale production of certain crops could become impossible. We would still have corn, wheat, potatoes and rice. But many fruits and vegetables we consume routinely today—such as apples, blueberries, broccoli and almonds—could become the food of kings.

Silent Bloom

When Hackenberg initially told us of his vanishing bees, our first thought was varroa mites. These aggressive parasites were largely responsible for a 45 percent drop in the number of managed bee colonies worldwide between 1987 (when they were first introduced in the U.S.) and 2006. Mature varroa females feed on hemolymph, the bees' blood. The mites also carry viruses and actively inhibit the hosts' immune responses. Hackenberg, like most expert beekeepers, already had long experience fighting mites, and he was adamant that, this time, the symptoms were different.

One of us (vanEngelsdorp) performed autopsies on Hackenberg's remaining insects and found symptoms never observed before, such as scar tissue in the internal organs. Initial tests also detected some of the usual suspects in bee disease. In the gut contents we found spores of nosema, single-celled fungal parasites that can cause bee dysentery. The spore counts in these and in subsequent samples, however, were not high enough to explain the losses. Molecular analysis of Hackenberg's bees, performed by the other of us (Cox-Foster), also revealed surprising levels of viral infections of various known types. But no single pathogen found in the insects could explain the scale of the disappearance.

In other words, the bees were all sick, but each colony seemed to suffer from a different combination of diseases. We hypothesized that something had compromised the bees' immune system, making them susceptible to any number of infections that healthy colonies would normally fend off. And Hackenberg was right: the prime suspects, varroa mites, were not present in numbers significant enough to explain the sudden die-off.

In the spring of 2007 our task force began detailed, countrywide surveys of all aspects of colony management, interviewing operators who had encountered CCD as well as those who had not. These and subsequent investigations ruled out several potential causes. No single beekeeping management method could be blamed. Large commercial beekeepers were as likely to suffer from high losses as were small operations or hobbyists. The symptoms affected stationary beekeepers as well as migratory ones. Even some organic beekeepers were affected.

As media reports of the die-offs surfaced, the public also started expressing concern. Many were eager to share their ideas as to the underlying cause. Some of these proposals—such as blaming CCD on radiation from cell phones—originated from poorly designed studies. Other hypotheses were untestable at best, such as claims that the bees were being abducted by aliens.

One theory favored by many concerned citizens was that bees could have been poisoned by pollen from genetically modified crops, specifically the so-called Bt crops. Bt crops contain a gene for an insecticidal toxin produced by the bacterium *Bacillus thuringiensis*. When pest caterpillars feed on crops producing these toxins, they die. But already before the onset of CCD, research had shown that the Bt toxin becomes activated only in the guts of caterpillars, mosquitoes and some beetles. The digestive tracts of honeybees and of many other insects do not allow Bt to work.

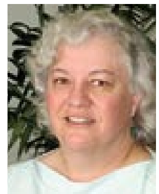
Another popular theory, and a more credible one, blamed synthetic poisons. The two main suspects were acaricides—chemicals beekeepers use to keep mites in check—and pesticides, either in the environment or in the very field crops the bees were pollinating. By 2006 newer types of pesticides had replaced older varieties. One type in particular, the neonicotinoids, had been blamed by beekeepers in France and elsewhere for harming insect pollinators. This class of insecticides mimics the effects of nicotine—a natural defense that tobacco plants deploy against leaf-eating pests—and is more toxic to insects than it is to vertebrates. But neonicotinoids also enter the pollen and nectar of the plant—not just the leaves—thus potentially affecting pollinators. Previous research had demonstrated that neonicotinoids decrease honeybees' ability to remember how to get back to their hive, a sign that they could be a contributor to CCD.

We and other experts also suspected that the bees' natural defenses might be undermined by poor nutrition. Honeybees—and wild pollinators, too—no longer have the same number or variety of flowers available to them because we humans have tried to "neaten" our environments. We have, for example, planted huge expanses of crops without weedy, flower-filled borders or fencerows. We maintain large green lawns free of any "weeds" such as clover or dandelions. Even our roadsides and parks reflect our desire to keep things neat and weed-free. But to bees and other pollinators, green lawns look like deserts. The diets of honeybees that pollinate large acreages of one crop may lack important nutrients, compared with those of pollinators that feed from multiple sources, as would be typical of the natural environment. Beekeepers have attempted to manage these concerns by developing protein supplements to feed colonies—although the supplements have not on their own prevented CCD.

All-Out Effort

Our task force focused its investigation on these two broad areas—pesticides and nutrition—in

[THE AUTHORS]

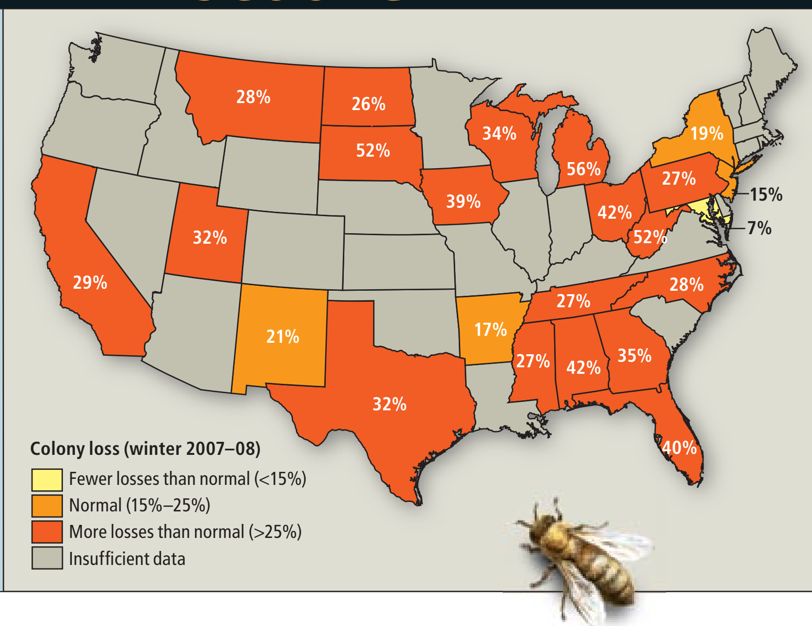



Diana Cox-Foster is professor of entomology at Pennsylvania State University and co-director of the colony collapse disorder working team, made up of experts from government and academia. Her research focuses on host-pathogen interactions. Cox-Foster traces her affinity for honeybees back to her great-grandmother, who was a commercial beekeeper in Colorado in the early 1900s. **Dennis vanEngelsdorp**'s passion for bees began in an undergraduate beekeeping course at the University of Guelph in Ontario. It has carried him through several appointments on West Indian projects and to his current dual position as acting state apiarist for the Commonwealth of Pennsylvania and senior extension associate with the entomology department at Penn State.

[THE EXTENT OF COLONY LOSS]

A COUNTRYWIDE SCOURGE

Colony collapse disorder (CCD) returned for a second year in the winter of 2007–08. A survey of beekeepers in the spring of 2008 asked how many colonies failed to make it through that winter. Nationwide 36 percent of colonies were lost (compared with a typical winter decline of 15 to 25 percent); 60 percent of those losses were attributed to CCD. Most states for which enough data were available were severely hit. Large losses were also reported in Australia, Brazil, Canada, China and Europe.



Number of honeybee colonies estimated to have died in the U.S. over the winter of 2007–08:

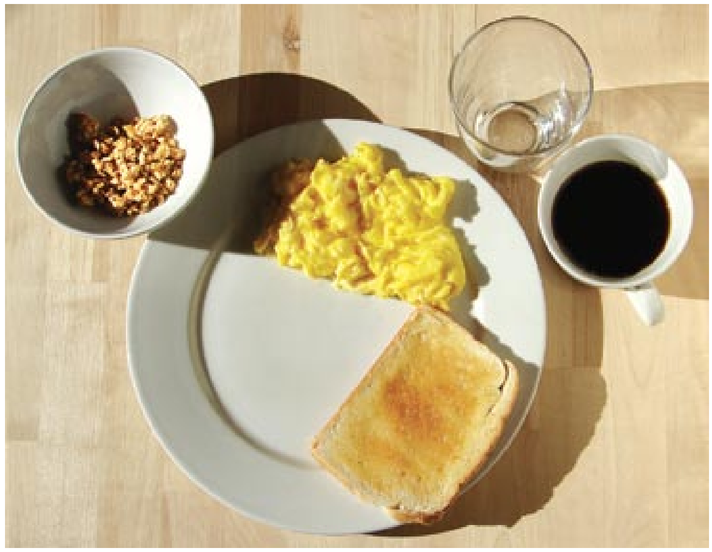
750,000 to 1 million

Some beekeepers reported losing up to

90%

of their colonies.

COURTESY OF ELLEN HARASIMOWICZ (*vanEngelsdorp*); COURTESY OF ELLEN JOHNSON (*Cox-Foster*); JEN CHRISTIANSEN, SOURCE: "A SURVEY OF HONEY BEE COLONY LOSSES IN THE U.S., FALL 2007 TO SPRING 2008," BY D. VANENGELSDORP ET AL., IN *PLOS ONE*, VOL. 3, ISSUE 12, ARTICLE E4071; DECEMBER 2008 (*map*)

WITHOUT HONEYBEES, many foods included in the breakfast at the left would become too rare for most people to afford. Shortages would affect an array of fruits, as well as jams and jellies, almonds and even milk, because dairies use alfalfa (which needs pollinators) as a protein-rich feed for dairy cows.

FAST FACTS

- There are an estimated 900 to 1,000 commercial beekeepers in the U.S., managing 2.4 million colonies.
- Nearly 100 kinds of crops require pollination by honeybees. The annual value of bees' work is $14 billion in the U.S. and $215 billion worldwide.
- Every February virtually all movable U.S. hives are taken to California to pollinate almond trees.
- Even before CCD, in certain regions of China bees had completely disappeared, possibly because of pesticide use, forcing orchard owners to pollinate pear trees by hand.

addition to the other obvious possibility, that a new or newly mutated pathogen could be causing CCD. Tests of our three hypotheses required collecting samples—lots of samples. We joined Jeff Pettis of the U.S. Department of Agriculture lab in Beltsville, Md., to conduct this monumental effort that involved long days, lots of miles on the road and the challenge of collecting enough material to share with the entire team. With no dead bees to study, we decided to collect live bees from apiaries in the midst of collapse, based on the premise that survivors would harbor the disease in its early stages. Bees were collected in alcohol for varroa and nosema counts. Bees, pollen and honeycomb wax were frozen on dry ice and rapidly shipped back to labs in Pennsylvania or Maryland to be stored in ultracold freezers and preserved for molecular and chemical analyses.

Some samples were sent to our colleague David Tarpy of North Carolina State University, who measured protein content. Tarpy found no notable difference between apiaries that had CCD and others that were seemingly healthy. His results suggested that nutritional state—on its own—could not explain CCD.

Much more startling was the outcome of our team's search for pesticides, for which we enlisted the help of Pennsylvania State University researchers Maryann Frazier, Jim Frazier and Chris Mullin and of Roger Simonds, a chemist at the USDA lab in Gastonia, N.C. (By coincidence, Simonds happens to be a beekeeper himself.) His broad-spectrum analysis, sensitive to insecticides, herbicides and fungicides, found more than 170 different chemicals. Most stored-pollen samples contained five or more different compounds, and some contained as many as 35. But although both the levels and the diversity of chemicals are of concern, none is likely to be the sole smoking gun behind CCD: healthy colonies sometimes have higher levels of some chemicals than colonies suffering from CCD.

No neonicotinoids were found in the original samples. But these or other pesticides cannot yet be exonerated. Honeybee colonies are dynamic, and our initial sampling was not—we took samples only once. It remains possible, if not likely, that bees afflicted by CCD were harmed by a chemical or mixture of chemicals not evident at the time we collected samples.

Our attempts to identify a new infectious disease—or a new strain of an old one—that could be at the root of CCD initially looked as if they would go nowhere fast. None of the known bacterial, fungal or viral diseases of bees could account for the CCD losses, so we had no clue what to look for.

Then Cox-Foster, with Ian Lipkin's group at Columbia University (and with help from biotech company 454 Life Sciences in Branford, Conn.), turned to a sophisticated microbe-hunting method called metagenomics. In this technique, nucleic acids (DNA and RNA) are collected from an environment containing many different organisms. The genetic material is all blended together and minced into pieces short enough that their sequences of code "letters" can be deciphered. In ordinary gene sequencing, researchers would then use computer software to put the pieces back together and reconstruct the genome of the original organism. But in metagenomics, the genes belong to different organisms, and so sequenc-

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ing produces a snapshot of the sequences in a collection of organisms, including microscopic ones, in an ecosystem. Metagenomics has been used to survey environments such as seawater and soil, revealing a surprising diversity of microorganisms. But it can also be applied to detecting microorganisms hosted by a larger organism, living either as collaborators (in symbiosis) or as infections.

Naturally, most gene sequences in our samples were from the bees themselves. But those were easy to filter out because, fortunately, the honeybee genome had just been sequenced. Nonbee sequences were then matched to genetic sequences belonging to known organisms. Researchers with expertise in molecular analysis of organisms—including bacteria, fungi, parasites and viruses—joined our team to identify potential culprits.

The *CSI*-style investigation greatly expanded our general knowledge of honeybees. First, it showed that all samples (CCD and healthy) had eight different bacteria that had been described in two previous studies from other parts of the world. These findings strongly suggest that those bacteria may be symbionts, perhaps serving an essential role in bee biology such as aiding in digestion. We also found two nosema species, two other fungi and several bee viruses.

But one bee virus stood out, as it had never been identified in the U.S.: the Israeli acute paralysis virus, or IAPV. This pathogen was first described in 2004 by Ilan Sela of the Hebrew University of Jerusalem in the course of an effort to find out why bees were dying with paralytic seizures. In our initial sampling, IAPV was found in almost all—though not all—colonies with CCD symptoms and in only one operation that was not suffering from CCD. But such strong correlation was not proof that IAPV caused the disease. For example, CCD could have just made the bees exceptionally vulnerable to IAPV infection.

Case Closed?

From subsequent work on IAPV, we know that at least three different strains of the virus exist and that two of them infect bees in the U.S. One of the strains most likely arrived in colonies flown in from Australia in 2005 after the U.S. government lifted a ban on honeybee importation that had been in effect since 1922. (The almond industry lobbied to lift the ban to prevent a critical shortage of pollinators at blossom time.) The other strain probably showed up earlier and is quite different. Where that one came from is unknown; it may have been introduced by way of importation of royal jelly (a nutrient bees secrete to feed their larvae) or a pollen supplement, or it may have hitchhiked into the country on newly introduced pests of bees. The data also suggest that IAPV has existed in bees in other parts of the world for a while, developing into many different strains and possibly changing rapidly.

In an effort to settle the issue of IAPV's role, Cox-Foster experimented with healthy honeybees that had no previous exposure to the virus. Her team placed hives filled with bees into greenhouses and fed the insects sugary water laden with IAPV. Sure enough, the infection mimicked some symptoms of CCD. Within one or two weeks of exposure, the bees began to die, twitching with paralytic seizures on the ground. The bees were not dying near the hives, just as one would expect in CCD. So those findings seemed to support the notion that IAPV can

To bees and other pollinators, green lawns look like deserts.

Wild Pollinators Are Ailing, Too

BUMBLEBEE

DERRICK DITCHBURN www.dereilanatureinn.ca

Honeybees are not the only pollinators to have suffered population drops in recent years. A National Research Council (NRC) report in 2006 pointed to downhill trends in certain species of North American wild pollinators, including some insects but also bats and hummingbirds. These species may be suffering from some of the same man-made afflictions that make honeybees vulnerable to CCD, such as introduced diseases, pesticide poisoning and impoverished habitats, says the study's lead author, entomologist May Berenbaum of the University of Illinois.

The western bumblebee, for example, has disappeared from a region stretching from central California to British Columbia, probably killed off by *Nosema bombi,* a single-celled fungus microorganism, according to work by entomologist Robbin Thorp of the University of California, Davis. The fungus may have spread to the western bumblebees from European bumblebees that U.S. farmers have imported to assist in the pollination of tomatoes and other crops in greenhouses, he says.

A more recent study published in the January *Biological Conservation* looked at historical data from Illinois and found that four bumblebee species disappeared there between 1940 and 1960—a period that coincided with large-scale agricultural intensification in the state, with consequent loss of prairie, forest and wetland habitats.

Declines in a few species of pollinating bats and hummingbirds—to the point that some bats are at risk of extinction—might relate to habitat changes. Many of them overwinter in Mexico, and biologists are urging the preservation of "nectar corridors," where the animals can find flowers along their migration routes.

But biologists can monitor only so many pollinator species (an estimated 200,000 exist worldwide), and not much is known about the state of health for most of them, the NRC report warned. Several Web-based collaborations call on citizen-scientists' help. Volunteers take pictures of pollinators and submit them to the Web sites, where researchers identify species and take note of where they were seen.

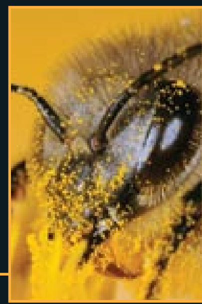
In 2008 the U.S. Congress for the first time modified its agricultural policy to include pollination protection measures, such as setting aside conservation land where wildflowers can grow and provide nectar. "That was a real landmark," Berenbaum says.

—Davide Castelvecchi, staff writer

[THE SEARCH FOR THE CAUSE]

Many Suspects, No Convictions Yet

Researchers have looked into virtually all aspects of honeybee life in search of the culprit behind colony collapse. The work has exonerated some suspects and has pointed to possible combinations of factors that can cause or contribute to CCD.



SUSPECT: CHEMICALS

As many as 170 different synthetic chemicals have been found in beehives of both sick and healthy colonies, with some samples of pollen stored in cells containing as many as 35 types. Although no single chemical seems to be the cause of CCD, pesticides may weaken bees' health.

SUSPECT: VARROA MITES

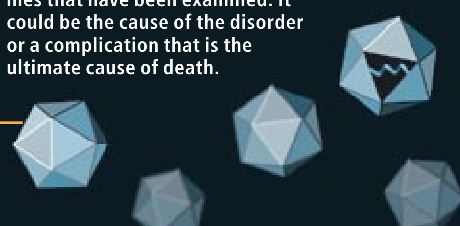
This mite, seen below sucking blood from a pupa (an intermediate stage between larva and adult), is the honeybee's most common and destructive pest. But collapsing colonies did not have significant mite infestations.

SUSPECT: PARASITES

Some of the bees in collapsing colonies were infected by single-celled fungi, such as *Nosema apis* (*below*), which invades the intestinal tract and causes dysentery. But levels of infection were too low to be lethal on their own.

SUSPECT: ISRAELI ACUTE PARALYSIS VIRUS

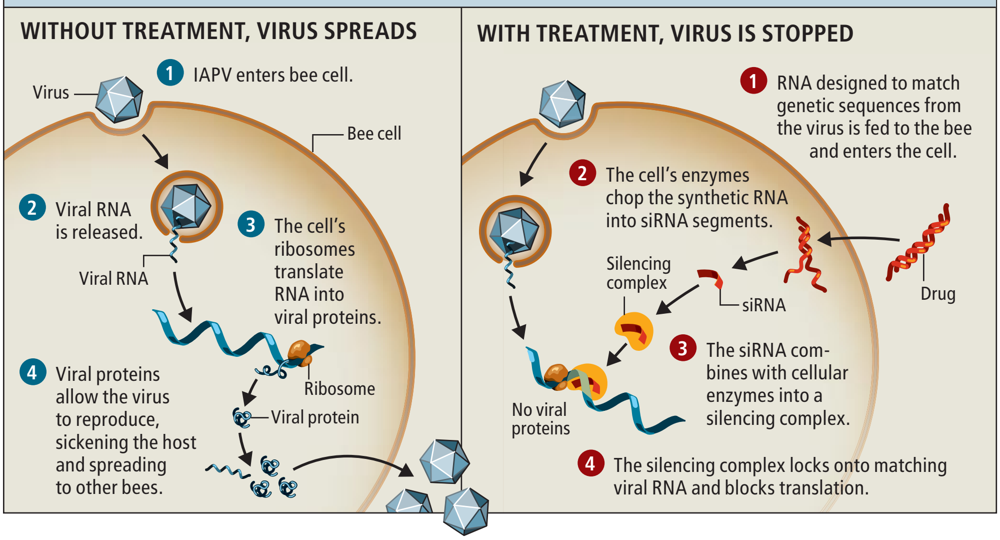
IAVP has been shown to produce symptoms similar to CCD's and has occurred in most affected colonies that have been examined. It could be the cause of the disorder or a complication that is the ultimate cause of death.



[ONE POTENTIAL SOLUTION]

A BEE MEDICINE?

A Miami-based biotechnology start-up company called Beeologics is developing an antiviral drug that exploits an ancient immune mechanism called RNA interference. Cells in most animals and plants use short-interfering RNA (siRNA) segments to inhibit the formation of viral proteins; here siRNA designed to target IAPV would be fed to colonies as part of double-stranded RNA mixed into a syrup.



cause CCD or at least contribute to the problem.

Additional sampling efforts by several groups showed, however, that IAPV was widespread in the U.S. and that not all infected colonies had symptoms of CCD, implying either that IAPV alone cannot cause the disease or that some bees are predisposed to be IAPV-resistant. In particular, a joint study the two of us initiated in 2007 with the USDA has tracked colonies owned by three traveling beekeepers and has observed colonies that were infected with IAPV without collapsing. Some of those colonies have later been able to rid themselves of the virus.

The growing consensus among researchers is that multiple factors—such as poor nutrition and exposure to pesticides—can interact to weaken colonies and make them susceptible to a virus-mediated collapse. In the case of our experiments in greenhouses, the stress of being confined to a relatively small space could have been enough to make colonies succumb to IAPV and die with CCD-like symptoms. More recent results from long-term monitoring have identified other unexpected factors for increased colony loss, including the fungicide chlorothalonil. Research is now focused on understanding how these factors relate to colony collapse.

A vaccine or cure for bee viruses and IAPV specifically would be desirable. Unfortunately, vaccines will not work on honeybees, because the invertebrate immune system does not generate the kind of protection against specific agents that vaccines induce in humans and other mammals. But researchers are beginning to pursue other approaches, such as one based on the new technique of RNA interference [*see box above*], which blocks a virus from reproducing inside a bee's cells. A longer-term solution will be to identify and breed virus-resistant honeybees. Such an effort could take years, though, perhaps too many to avoid having a large number of beekeepers go out of business.

Meanwhile many beekeepers have had some success at preventing colony loss by redoubling their efforts at improving their colonies' diets, keeping infections and parasites such as varroa and nosema in check, and practicing good hygiene. In particular, research has shown that sterilizing old beehive frames with gamma rays before reusing them cuts down the risk of colony collapse. And simple changes in agricultural practices such as breaking up monocultures with hedgerows could help restore balance in honeybees' diets, while providing nourishment to wild pollinators as well.

Humankind needs to act quickly to ensure that the ancient pact between flowers and pollinators stays intact, to safeguard our food supply and to protect our environment for generations to come. These efforts will ensure that bees continue to provide pollination and that our diets remain rich in the fruits and vegetables we now take for granted. ■

MORE WAYS TO FIGHT BACK

Restoring a balance to the habitat of pollinators might improve their general well-being and help prevent colony collapse. Large stretches of single crops or residential lawns could be broken up with more "weedy" meadows and hedgerows. Plants flowering at different times of the year could then provide more variety in pollinators' diets and support them year-round.

Sterilizing used beehives with DNA-destroying gamma rays before reusing them for a new colony cuts down the risk of CCD recurrence, possibly because it kills microorganisms that contribute to the disease.

Research on the impact of pesticides on pollinators usually focuses on possible lethal effects. More research is needed on whether certain pesticides can put insects under stress, even if the chemicals do not kill them outright.

➥ MORE TO EXPLORE

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The Mid-Atlantic Apiculture Research and Extension Consortium: **http://maarec.cas.psu.edu**

The Xerces Society for Invertebrate Conservation: **www.xerces.org**

OPPOSITE PAGE: ANDREW SYRED *Photo Researchers, Inc. (varroa mite)*; CENTRAL SCIENCE LABORATORY *Photo Researchers, Inc. (varroa mite on larva)*; HEIDI & HANS-JURGEN KOCH *Minden Pictures (bee with pollen)*; COURTESY OF BARTON SMITH, JR. *USDA Bee Research Laboratory (Nosema sp.)*; JEN CHRISTIANSEN *(Israeli acute paralysis virus)*; OPPOSITE PAGE AND THIS PAGE: JEN CHRISTIANSEN *(illustrations)*