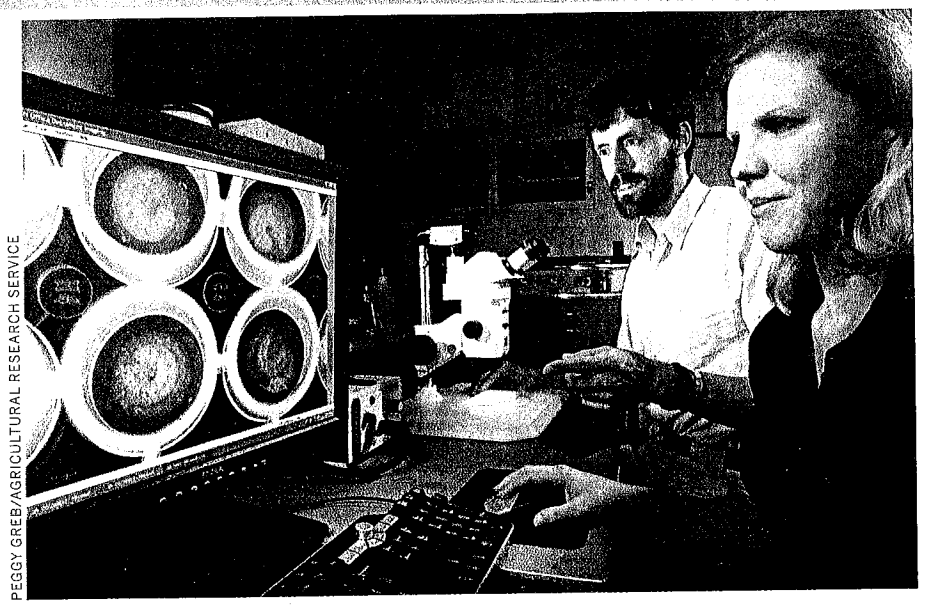

PEGGY GREB/AGRICULTURAL RESEARCH SERVICE

DIAGNOSIS HUNTERS Agricultural Research Service scientists Evans (left) and Dawn L. Lopez look for signs of disease in larval honeybees.

WHY ARE THE BEES DYING?

As bee **COLONIES COLLAPSE** across the U.S. and other countries, researchers search for causes

BETTE HILEMAN, C&EN WASHINGTON

THE DISAPPEARANCE of honeybee colonies was first reported this past October. Beekeepers along the East Coast became alarmed as they realized they were suddenly losing 30–90% of their colonies. The problem, now called colony collapse disorder (CCD), has spread across the U.S., and no one knows what's causing it.

By May, CCD had moved to 36 states, decimating a quarter of the nation's 2.4 million bee colonies. Nearly 30% of 577 beekeepers in the U.S. reported that CCD had affected their hives, and they had lost up to 75% of their colonies, says Jerry J. Bromenshenk, a biology professor at the University of Montana, Missoula, and chief executive officer of Bee Alert Technology, a technology-transfer firm associated with the university.

In the affected hives, nearly all the worker bees simply disappear. The queen bee and the immature bees in the pupa stage are essentially abandoned with only a handful of young bees to feed and care for them. Food stores—both honey and processed pollen—remain in the hives and are not robbed by other insects, such as wax moths. Rather than succumbing in the hive or on the ground around it as observed in other colony disorders, the worker bees hit by CCD simply abandon the colony and inevitably die because solitary honeybees cannot survive.

In January, Diana Cox-Foster, an entomologist at Pennsylvania State University, and Dennis vanEngelsdorp, state apiarist with the Pennsylvania Department of Agriculture, established a network of researchers made up of university and government scientists to study the problem. So far, this working group has been able to basically eliminate four possible causes: genetically modified crops, cell phone towers, parasitic varroa mites, and unusual weather pat-

terns. But other potential causes—pathogens, poor nutrition, and exposure to toxic substances such as insecticides—remain on the table. Some scientists believe that industrial-scale beekeeping, which involves feeding the bees sugar syrups and transporting hundreds of thousands of colonies around the country, puts too much stress on the insects' immune systems, making them more vulnerable to pathogens.

Honeybees are a big business in the U.S. They are responsible for the pollination of 95 kinds of fruits and vegetables and add $14.6 billion in extra yield and crop quality. Despite the colony losses since last October, this year's crops will not suffer, vanEngelsdorp says. Beekeepers, however, can't sustain large hive collapses two years in a row, he says. "Another bad year could put many beekeepers out of business and result in declines of crop yields or failures."

CCD has happened in the past. According to May R. Berenbaum, head of the entomology department at the University of Illinois, Urbana-Champaign, and chair of the National Research Council committee that wrote a report on pollinators last year, the literature mentions a similar phenomenon in the 18th century, another event in 1897, and several outbreaks in the 1960s and '70s. But the malady has not shown up before on such a large scale, and the details of some of the previous outbreaks were different, Berenbaum says.

BEEKEEPERS in the U.S. are not the only ones experiencing CCD. Many of those in Canada, France, Switzerland, Italy, Spain, and Portugal are also having problems, Berenbaum says, and Taiwan has been hit particularly hard.

Compared with other honeybee disorders, CCD has several unique symptoms not seen in past collapses associated with infections by mites and viruses. With CCD, few, if any, dead bees are found in the hive. Current collapses occur very rapidly, as worker bees abandon a colony within two weeks, sometimes in as little as two days. The few bees that remain in the hive eat little and have high levels of bacteria, fungi, and parasites in their guts. Usually, when a bee colony is failing, opportunistic insects such as wax moths or hive beetles invade and eat the stored honey. But in hives hit by CCD, the abandoned food stores are left untouched.

However, researchers do not know whether some kind of bacteria, fungus, or parasite is the primary cause of the U.S.

outbreak or whether these pathogens infect the bees after their immune systems have been suppressed by poor nutrition, pesticide exposure, or the stress of being moved long distances in tractor-trailers.

U.S. beekeepers have experienced other kinds of problems during the past decade. They suffered large losses starting in 1987, when varroa mites—parasites that attack young and adult bees—started invading hives and killing the bees. Varroa mites can devastate a hive within a few months and have wiped out nearly all the feral honeybees. In managed colonies, varroa mites can now be controlled with miticides or by placing a screened board with sticky paper in the bottom of the hive. That way, when mites become dislodged from the bees, as often happens, they fall through the screen and are caught on the sticky paper.

To solve the CCD mystery, research on various toxicants and pathogens is now being conducted at the U.S. Department of Agriculture's Agricultural Research Service, the Florida Department of Agriculture, and several universities.

Among toxicants, one of the primary suspects behind CCD is the insecticide imidacloprid, explains Jerry Hayes, chief of the apiary section of the Florida Department of Agriculture. "Imidacloprid certainly looks like it could be one cause of CCD just because of the way it kills other insects," he says. "It makes them disoriented and causes their immune systems to collapse. One way it causes death in termites, for example, is the insects go out to feed and then can't remember how to get home. It also makes them more susceptible to natural pathogens in their colonies."

JAY EVANS/AGRICULTURAL RESEARCH SERVICE

CAREFUL MOVES Entomologists check the status of a bee colony at the USDA-ARS Bee Research Lab in Beltsville, Md.

Imidacloprid is a relatively new chloro-nicotinyl insecticide that was first marketed for corn in France in 1992, for sunflower seeds in France in 1994, and in the U.S. in 1994. Imidacloprid is now registered in 120 countries and sold for use on more than 140 agricultural products. It is

also applied extensively to golf courses and home gardens. The compound has very low mammalian toxicity, but it is neurotoxic for insects. Bayer, the manufacturer, won't divulge sales or use figures for individual regions, but use in California has escalated enormously from 5,178 lb in 1994 to 163,168 lb in 2005, according to state statistics.

In 1995, after imidacloprid was first used on sunflower seeds in France, beekeepers there complained that the product was making bees so disoriented they could not find their way back to the hives. The National Union of French Beekeepers estimated that the number of hives plummeted from nearly 1.5 million in 1996 to 1 million in 2003, and that honey production fell from 75 kg to 30 kg per hive. Beekeepers in France, and in the rest of Europe, do not transport their hives to follow crops as they flower.

SEVERAL STUDIES, conducted in four different areas of France, did not find any differences in bee behavior, evolution of beehives, and honey harvest with and without imidacloprid. However, under pressure from beekeepers, the French Ministry of Agriculture & Fisheries banned imidacloprid in 1999 as a sunflower seed coating, and it renewed the ban in 2001 and again in 2003. The ministry banned the use of imidacloprid on corn in 2004 and outlawed the use of fipronil—another neurotoxic insecticide—in all crops in 2004.

When the bee colonies that farmers in France used to pollinate sunflowers continued declining after imidacloprid was banned, beekeepers blamed the problem on insecticide residues in the soil where treated seeds had been sown previously and on the replacement insecticide fipronil. In 2005, honey harvests in France improved for the first time since bans on imidacloprid and fipronil were imposed. However, CCD problems still exist in many parts of rural France.

A similar battle over imidacloprid is going on in Canada's Prince Edward Island, where the insecticide is used on potatoes. Beekeepers there say the product is responsible for losses in hives needed for blueberry pollination.

David Fischer, head of the ecotoxicology section at Bayer CropScience, insists that imidacloprid as a seed coating is not dangerous for honeybees. According to Bayer's and other studies, he says, the lowest level of imidacloprid that could cause an effect on honeybees is 20 ppb. When imidacloprid is used to coat seeds, the residues of the product in the pollen and nectar are never higher than 5 ppb, a level that "poses only a negligible risk to honeybees," he explains. This conclusion is based on more than 30 studies conducted by Bayer and universities in various regions.

Despite all of those negative results, efforts to show that imidacloprid and other pesticides are a cause of CCD continue. Hayes and his colleagues are analyzing beeswax and beebread—the pollen modified by the bees into a food source—from affected and unaffected colonies for toxicants.

"Beeswax, a fatty acid, is a chemical sponge that absorbs a lot of things bees bring back to the colonies," Hayes says. "It gives you a record of everything the insects have come in contact with." The data are not

yet available to show whether any pesticide is found at higher levels in affected colonies.

Hayes, believes, however, that CCD probably has more than one cause. The poor nutrition that comes from eating only one type of pollen, such as that on almond trees, for several weeks at a time; the artificial diet of sugar syrup given to boost bee populations early in the growing season; the antibiotics administered to sick bees; and the stress of traveling are also part of the mix, he says. "One of the confusing and frustrating things about CCD is that there are some tantalizing tidbits out there, but all of the collapsed hives don't seem to share a single common factor that could cause the problem," he notes.

VanEngelsdorp is also involved in broad screening of affected and unaffected hives for about 180 pesticides and other chemicals that might be contributing to CCD.

IN CONTRAST, Jay D. Evans, research entomologist at USDA's Agricultural Research Service, Beltsville, Md., is focusing on pathogens as a CCD trigger. "There is evidence that the cause is a contagious agent of some sort, instead of a pesticide or some other stress," he says. He is performing a genetic survey of sick and healthy colonies. To find pathogens, Evans uses polymerase chain reactions to amplify specific chunks of DNA to determine if they are from viruses, bacteria, or fungi. Out of 50 possible pathogens, Evans and his collaborators have narrowed the list to four or five. They are studying each one on the short list to see if it is a reasonable cause. One of the questions the researchers ask in each case is this: "Is it new to North America or has it spread a great deal in the past couple of years?"

Evans believes CCD first showed up extensively in hives that had been moved to California to pollinate the almond trees and then spread out from there. Early each year about half the hives in the nation are taken to California for use in the almond groves when they bloom in February, he explains. After that, many of those same hives are moved north into Washington for apple tree pollination, some remain in California for later crops, and some are hauled to the Dakotas for honey production, he says. Consequently, if some of the almost 1 million hives that were transported to California for the almond pollination early this year carried a new pathogen, that would have provided an excellent mechanism for spreading it around the U.S., he says.

Evans is also using a panel of the known honeybee immune genes to compare the activity levels of genes associated with the immune systems of bees in collapsing colonies with those of healthy bees. If, in fact, the bees from collapsing hives are immunocompromised, there should be an indication of that in the expression levels of these genes.

On the other hand, if the bees from collapsed colonies have been exposed to a pathogen, specific immunodefense genes should be activated. In fact, the signals of this immune response may provide clues into the specific class of pathogens present. So Evans is examining those genes as well. None of this research would be possible without the honeybee genome, which was elucidated and published last year by a consortium headed by Gene Robinson, who heads the bee research facility at the

PEGGY GREB/AGRICULTURAL RESEARCH SERVICE

BEE GENES Worker honeybee perches on a gel image showing bee DNA.

University of Illinois, Urbana-Champaign.

Berenbaum and Robinson are also using gene chips to look at the activity of genes associated with chemical detoxification. "We are looking for a footprint of exposure," Berenbaum says. Their work is generating an enormous amount of data that won't be analyzed until the end of the summer at the earliest. "Fortunately, thanks to the sequencing of the honeybee genome, we now have 21st-century tools to use to search for the causes of CCD. Knowing the sequence of the genome has allowed us to design a gene chip that contains all the known honeybee genes, so we can monitor their activity in healthy bees and in bees either from affected colonies or colonies nearby," Robinson says.

One genetic detail of honeybees that makes them vulnerable to toxicants is that they have only half the number of genes to detoxify foreign substances that other insects have, and these genes don't turn on very easily, Berenbaum explains. This appears to be related to the bees' social behavior. Because the whole hive functions as a unit, if one worker dies from some kind of exposure, this makes little difference to the colony as a whole.

Despite their new tools, researchers may not find the cause of CCD, Berenbaum says. "It is not outside the realm of possibility that the current CCD episode will pass, just as other incidents have passed, and we still won't know what happened," she says. "The trouble with honeybees is they are complicated, and this makes investigating them really complicated. A honeybee by itself is a living, breathing organism that has physiology and behavior, but it's very difficult to interpret those behaviors outside the colony. Nevertheless, I'm optimistic that with the incredible new knowledge, we can figure out what is going on." ■