Sixty-Eight Researchers
Gathered Under One Cover
Explain "What We Know Today"

Honey Bee Health; Challenges and Sustainable Solutions is a collection of current scientific studies from Europe and the Americas.

By M.E.A. McNeil

"'Where can I find out *more* -- about viruses or whatever?' Beekeepers were asking me, " said USDA researcher Diana Sammataro. She didn't have a good answer. "It's very difficult if you're a beekeeper to get this information. You get it in little fits and starts."

Access to scientific journals in academic libraries is a tricky business unless you are faculty; or you can cough up hundreds of dollars for a subscription to just one of the dozen or so scholarly publications that deal with bees in one way or another. Even well-established researchers such as Sammataro can have a hard time with the profusion of specialization: She and Jay Yoder, of Wittenberg University in Ohio, did a study on beneficial (helpful) fungi in bee bread that fit neither a scientific bee journal (because it was about fungi) nor a scientific fungus journal (because it was about bees); for a European journal, it was an American problem.

"Why don't we do our own book and include current research being done on honey bees?" Yoder asked. It was a simple but ambitious solution to the need for accessible reference material on current bee science.

"All those little pieces of information," said Sammataro. "We wanted to bring them together, to say: This is what we know about chalk brood now, and this is what we know about nosema now, what we know about varroa.

"We came up with a plan with some chapter headings. "I emailed some colleagues and asked, 'Would you be interested?' The response was completely a surprise; the feedback has been phenomenal. People got back with what they wanted to write about. That's what shaped the book. It was all organic -- letting people who are experts in their own area write about their work."

Honey Bee Colony Health; Challenges and Sustainable Solutions (Taylor & Francis, 2012) is comprised of 21 chapters by 68 American, Canadian, South American and European researchers, with many trans-Atlantic collaborations. "It is not light reading and not meant to be," said Sammataro. It is not a how-to on beekeeping: "It is meant to be a reference book." At $85, it may not be a casual purchase, but it is a valuable choice for a bee library – especially a college or club collection. The contents, determined by the responses, turned out to cover nearly the range of current concerns, among them: pests and pathogens, the effects of various toxicants on bees, and scientific forensic methods. They all add that "Little is known about…" whatever it is that would be great to learn next; but they are scientists, and that is part of the gig. They have, though, ferreted out a lot and have come to some agreements: no one thing is bringing the bees down and, however nuanced the situation, pesticides are not doing the bees any good.

The lay reader can skirt sections of the material clearly intended for working scientists that give, for example, detailed instructions for doing molecular analysis or formulae for assaying surveys. The majority of the material is accessible and enormously informative. The book, replete with photos, is laid out with a unifying color scheme that makes its many charts and diagrams inviting. Eric Mussen, UC Davis Extension Apiarist, noted, "Many chapters contain data and information that we have seen reported at meetings, but could not previously find in print."

The articles in the book were peer reviewed in various ways: for example, through publication in scientific journals or in-house vetting for USDA researchers. Also, as the chapters arrived, Sammataro, Dewey Caron and Ann Harmon each reviewed them as did the science editors at the publisher.

Contributors deal in various ways with why honey bees are in crisis, what questions are raised, and what action to take. What follows are some samples from a book that goes far deeper into each of these subjects:

The Problem

Dennis van Engelsdorp, together with a consortium of 24 others report on how they are going about assaying information on the wellbeing of honey bees. Their Bee Informed Partnership employs a two-part process: gathering data from beekeepers, then calculating and reporting. It is a complex undertaking, given the dynamic nature of an apiary, and, he says, it begs to be standardized. Van Engelsdorp offers statistical methods to that end and embraces the international adoption of a model winter loss questionnaire created by an international network of bee researchers with the acronym CLOSS (Prevention of Honey Bee Colony Losses).

We have "good reason for alarm," writes Keith Delaplane of the University of Georgia of the decline of all pollinators. Although many native bee species have efficient pollinating behaviors, they are often specialists with a relatively brief period of activity; honey bees are generalists, field a large forager force, and have a long active season. "This benefit goes beyond the mere dollars and cents contributed by bees to agricultural production (estimated at over $200 billion worldwide) to the point that bees are determiners of the diversity and quality of human diets." He reports that even though there are areas of managed honey bees that are stable in Africa, Australia, South America and some parts of Europe, "The number of managed beehives is not keeping pace with the global demand for pollination services these bees provide." He writes that entire issues of scientific journals have been dedicated to the decline of the bees (*Journal of Apicultural Research*, January 2010; *Journal of Invertebrate Pathology*, January 2010; *Apidologie*, June 2010). Good thing we have this book to tell us what they say.

Delaplane offers an informative description of the relation of pollination to floral morphology: canola, for example, is morphologically simple enough to be accessible to a broad array of visitors, whereas the blueberry flower is so complex that it must be actively vibrated by a bee – a behavior called sonication. Although it appears that honey bee disorders impact pollination by outright killing colonies, he posits that a more nuanced understanding of how that happens will better inform management decisions.

A new direction in investigating honey bee mortality is described by Yves Le Conte, the director of the bee lab at INRA, the French National Institute for Agricultural Research. Since no single cause of decline has been found among the many biotic and abiotic factors bees are exposed to, studies analyzing the interactions among risk factors are emerging. Bee physiology, pathogen loads and pesticide levels are among 61 variables being investigated.

Le Conte explains that the honey bee genome is deficient in genes coding for detoxification enzymes. Because a large variety of pesticide residues are found in the hives, it was thought that the bees are endangered by these chemicals -- but decline has not been traced to any one pesticide. Most studies on the impact of pesticides on honey bee health have focused on one chemical at a time; now the combined effects of multiple toxins are suspected to significantly affect the bees. It is a major investigatory challenge to experimental design because of the enormous number of molecules involved. LeConte gives an overview of contributing interactions with pesticides that include pathogens, genetic diversity and nutrition.

Conservation biologist David Inouye, of the University of Maryland, discusses ecological influences on decline. He adds to the discussion climate change, which can alter the phenology of flowering to affect all bees. Where there are data, a reduction in floral sources has been recorded, however there is still a surprising paucity of information about the abundance and distribution of native pollinator species. In the US, for example, four species of bumblebee have declined nearly 96% and their geographic ranges contracted by 23 to 87%. In addition to habitat fragmentation and loss, the introduction of a destructive protozoan pathogen, *Crithidia bombi* has been attributed to commercial bumble bees used for pollination in greenhouses. "Our understanding of the consequences of these threats is limited by our lack of knowledge of the distribution and abundance of pollinator populations. Data collection by both scientists and citizen scientists is helping to solve this problem," writes Inouye.

Pests and Pathogens

Who knew that there are over 30 species of mites associated with honey bees? Well, Sammataro for one; she writes a fascinating survey of the extraordinary diversity of mites, describing some that are predatory, incidental, facultative, obligatory or phoretic. Many are simply freeloaders, feeding on hive products such as bee bread. But three obligate parasitic mites have caused massive losses and increased mite-associated pathologies, mostly viruses: varroa, tracheal mites and Tropilaelaps, a virulent species not yet reported in the Americas.

Tropilaelaps is considered to be an emerging global threat to beekeeping, not only for its injury to bees but because it also carries the deadly deformed wing virus. No one knows what would happen if it arrives here, but the hope is that, because it cannot feed on adults, it may not survive in temperate climates with a long broodless period.

Varroa, however, is still the major topic of concern. Sammataro writes that over 1700 journal articles on Varroa have been published since 1971 compared to 102 articles on tracheal mites since 1934. Tropilaelaps appears in 28.

Some management recommendations appear throughout the book in relation to the science. For example, Sammataro counsels biannual mite testing, spring and fall, and more if mite populations are growing. "Fewer treatments save money, diminish the buildup of resistance to acaricides, and lessen contamination in the hive and its products," she writes, giving practical directions for various testing methods. A chart listing chemotherapies for mite control together with studies and results are informative (e.g., small cell size does not work). She concludes that IPM methods are best for reducing chemical treatments and lobbies for new strategies.

Ingemar Fries of the Swedish University of Agricultural Sciences writes, "Efficient mite control masks any differences in tolerance leading to a continuous need for mite control." The BEEDOC project, a collaborative effort of Swedish researchers with honey bee breeders, is working on a project to distinguish colonies with more varroa mite resistance. Fries has limited selection criteria to two characteristics that are relatively easy to measure: hygienic behavior and mite population growth rate.

He writes that ultimately mite tolerance will likely be a combination of qualities that may vary in different geographic areas. At this point, often the bees that survive through natural selection can lose desirable properties for profitable beekeeping.

Swedish and American bee virus experts Joachim de Miranda and Yan Pin Chen teamed to write a worthwhile instructional text on what viruses are –""the ultimate parasites, entirely dependent on their hosts for reproduction." Viruses even have their own molecular parasites that can alter the symptoms of their host virus. Many viruses persist and spread harmlessly at low titers within and between bee populations.

Chen and de Miranda discuss diagnosis: symptoms provide the cheapest, easiest, and quickest evidence, but only a few viral diseases are unambiguous -- sacbrood, black queen cell and deformed wing viruses. The problem with diagnosing by symptoms is that they appear only at very high titers. Forensic diagnostic tools are explained, with the researchers concluding that PCR (polymerase chain reaction) is the best. What is known about transmission of bee viruses is shown in a clear chart: by contact, vectorborne (e.g. by Varroa), vertical transmission (e.g. eggs), venereal (e.g. through sperm).

Chalkbrood fungus is another pathogen always present in bee colonies at a low level without causing disease symptoms, according to USDA researchers Katherine Aronstein and Humberto Cabanillas. It requires predisposing conditions to develop, for example, cool, moist weather. A broad range of chemotherapeutic compounds have been tested with none achieving a level of control required to fight this disease. Currently there are no antifungal compounds registered for use against chalk brood in bee colonies, and attempts to find natural products for microorganisms to control it have not produced usable applications. The best control is prevention: young, hygienic queens, good nutrition, clean equipment, good ventilation, regular replacement of frames. Antifungal activity in bee bread is an important natural deterrent.

Healthy, strong bees are the best defense against small hive beetle, too, according to Jamie Ellis of the University of Florida. The beetles, which have become more of a regional problem, exact a heavy price from the bees: not only do they feed on honey, pollen and brood, but they carry sacbrood virus, deformed wing virus, and American foulbrood bacteria. "Because of the lack of quality chemical control options, attention has focused on integrated pest management," writes Ellis. In addition to available, moderately-effective hive trapping devices, he describes experimental development of a yeast and soil-dwelling nematodes as biological controls.

Pesticides and Fungicides

A group of experts on the effect of chemicals on bees, Reed Johnson and Marion Ellis of the University of Nebraska, Christopher Mullin and Maryann Frazier of Penn State University, concur with others that no single pesticide has been shown to be responsible for the decline of honey bees. However, "chronic exposures to neurotoxic insecticides and their combinations with other pesticides, in particular fungicides, are known to elicit reductions in honey bee fitness." They cite 121different pesticides and metabolites found in 887 samples from migratory and stationary beekeepers. Although systemic pesticides, such as neonicotinoids, minimize direct exposure to bees during application, the bees may instead be exposed to these pesticides or their metabolites in pollen, nectar, and plant exudates over extended periods of time. The effects of chronic exposure to pyrethroids, organophosphates, neonicotinoids, fungicides, and other pesticides can range from lethal to sublethal effects in the larva and workers to reproductive effects on the queen. These chemicals may act in concert in ways currently unknown to create a toxic environment for the honey bee.

"Beekeepers researching the primary source of pesticides contaminating beehives need only to look in a mirror," they write. All wax foundation samples from North America are contaminated with tau-fluvalinate, coumaphos and pesticide metabolites – all placed in hives for control of varroa and lingering with a long half-life. "The uniform presence of these acaricides in foundation is particularly disturbing because replacement of frames is the main avenue currently used to purge colony of accumulated pesticide contaminants."

The researchers call for a change in the regulatory system "to make effective and safe veterinary pesticides available to beekeepers."

Brazilian and Slovenian researchers, Elaine Silva-Zacarin, Roberta Nocelli and Ales Gregorc, contributed a chapter on what is known about resistance mechanisms to toxins in honey bees. The most widely studied are behavioral, physiological, morphological, and biochemical. They delve into description of organs and tissues of bees as targets of pesticides down to response on a cellular level. When conventional protocols are not sufficient to answer questions about the effects of sublethal doses over the long-term, they find that analysis of morphological changes in target organs of bees – cellular stress and cell death – are important markers.

Biological control of bee pests is a small but growing field as beekeepers and bee researchers seek ways to reduce pesticide use. Two approaches are used: With classical biological control a new organism, parasite or a pathogen is released into an ecosystem where it did not previously occur in order to control a particular pest. With augmentative biological control, the natural enemy to be used already exists in the ecosystem but at a density too low to sufficiently control the target pest. William Meikle of the USDA describes research underway for biological controls against wax moths, varroa, and small hive beetle.

Increased reports of chalkbrood in bee colonies regularly moved for pollination piqued the interest of researchers, who knew that beneficial fungi serve as a natural defense against chalkbrood and stonebrood. The result is the fascinating chapter by Yoder, Sammataro and Gloria DeGrandi-Hoffman on the relationship between fungicide exposure and the fungi that bees use to process and store bee bread.

Pollen becomes bee bread through fermentation by microorganisms, transforming it in chemical composition. Bee bread activates the hypopharyngeal glands of nurse bees for the feeding for larva and the queen. Numerous species of fungi -- including yeasts and molds – have been identified in bee bread, and they are responsible for the synthesis of vitamins, enzymes, and other compounds that aid in digestion and pollen preservation. Some inhibit the growth of other fungi, presumably to keep a healthy balance.

California bee bread samples showed a pronounced reduction in overall fungi quality, and lab results reflected occurrence in the field: The microflora was found to be modified both in the area were bees collect pollen as well as inside the colony. "Because of reduced fungus quality it is conceivable the key metabolites needed by bees for food processing and preservation may be absent. The end result could be a chronic weakening of the colony from malnutrition." In addition, some fungicides cause bees to become disoriented during foraging or can be lethal, according to studies mentioned in the chapter.

Microbes

Inferring from human studies, the wellbeing of all organisms depends on maintaining conditions that encourage microbial growth. “We are just beginning to learn about the role of microbes in the health of all organisms, including honey bees. We know that many metabolic processes are not directed by host genes but rather by those symbiotic microbes,” writes DeGrandi-Hoffman with Bruce Eckholm and Kirk Anderson of the USDA.

Turkish researcher Asli Ozkirim finds that microflora in bees have seasonal fluctuations – especially commensal organisms that compete with pathogens and prevent their colonization. His studies of the bacteria in the bee gut leads him to recommend development of bacterial approaches to disease control to avoid commercial antibiotics.

Genetics

Susan Cobey and Steve Shepherd of Washington State University, with David Tarpy of North Carolina State University, come to a surprising conclusion about honey bee queens: Studies of queen quality, in 10 to 20 year intervals beginning in1947, do not support the overall perception that queens are now poorer. Their condition appears not to be drastically diminished from historical levels. Still, beekeepers are reporting such problems as premature supercedure and inconsistent brood patterns.

The researchers describe three historical genetic bottleneck events: the limited original importation of European stock, ongoing commercial breeding from a restricted number of queens, and the destructive invasion of foreign parasites.

Microbreeder programs, locally adapted colonies among small scale beekeepers attempting to create survivor stock, are often based on the collection of swarms. “Results are often unpredictable and disappointing due to a lack of rigorous selection criteria and controlled mating.”

Because research breeding programs usually have short-term funding, they have often been turned over to beekeepers without oversight. “Without a long-term commitment in supporting resources, selection efforts are relaxed and gains are quickly lost,” they write. The situation is changing; testing mating stock for multiple characteristics, including hygienic behavior, is becoming more common. “Industry support will determine the viability of such programs.”

They conclude that the quality of queens can be significantly improved by addressing the genetic bottlenecks in our breeding systems and increasing the overall genetic diversity of the honey bee population. “Development, maintenance, and adoption of highly productive European honey bees stocks that can both tolerate varroa and resist diseases offer a sustainable, long-term solution to these ongoing problems. Developing such a suite of traits has been elusive. Bee breeding is subject to unique challenges, including high labor costs, slow progress toward breeding goals, and little economic profit.”

Forensics

The use of molecular traits to identify parasites and pathogens is a developing front in diagnosing bee diseases. These genetic-based tools, described by Jay Evans, I. Barton Smith, Jeff Pettis and Judy Chen of the USDA, allow sensitive detection of microbes. Genetic markers have now been identified for the major pests and diseases. In addition, readable genetic tags have been found for bee proteins involved with development, immunity, physiology, and behavior. A chart showing the cost for Quantitative PCR on a sample tested for eight bee targets comes to under $19.23, but, like potato chips, you can’t stop at just one.

An electron microscope has been required to tell the difference between *Nosema apis* and *Nosema ceranae,* according to Richard Fell and Brenna Traver of the Virginia Polytechnic Institute. The difficulty in distinguishing species based on spores has led to the use of PCR as the most widely used molecular detection technique.

Antibodies specific to *N. ceranae* can also be used for identification, according to Thomas Webster of Kentucky State University and Katherine Aronstein of the USDA. A test can detect infestation at as few as 1000 spores, a tiny fraction of those present in a highly infected bee. The USDA Office of Technology Transfer is working toward developing a sample test that would not require any laboratory equipment and can be used directly in the field. The researchers hope to see this test commercially available to beekeepers.

New Research

A recent COLOSS workshop in Switzerland reported research priorities as: biological controls, trigger of varroa reproduction, development of varroa *in vitro*, search for varroa tolerant bees and identification of tolerance mechanisms, host parasite co-evolution, new invasive mites, *Varroa destructor* genome, modeling of population dynamics, mite virulence thresholds, virus transmission and virulence. The researchers in this book add: chemical interactions, effects of sublethal chemical exposure, the role of microbes, broader effects of supplements, the development of probiotics.

The list would seem overwhelming if it were not spawned in an era of rapid growth in biotechnology, shared information, and a desire of beekeepers to strengthen the bees. Citizen science projects, listed in the book, generate data of value for scientists. The LD50 of many pesticide compounds are now available to the public at: www.ipmcenters.org/Ecotox/DataAccess.cfm.

“Preventing problems is always easier than solving them,” write DeGrandi-Hoffman, Eckholm and Anderson. “In the case of developing beekeeping practices to prevent colony losses, perhaps the best solutions reside in the bees themselves.”

If the book offers a conclusion for beekeepers, it is: Stay ahead of the problems, keep your bees healthy, and stay tuned.

#

Note: A patchwork of support made the research reported in this book possible: The California State Beekeepers Association, The Almond Board of California, The BeeDoc Research Network, USDA CAP Grant, INRA SPE, CONICYT/French Embassy of Chile Grant, The Florida State Beekeepers, The Tampa Bay Beekeepers, Penn State College of Agriculture Sciences, and Preservation *Apis mellifera* (P*Am*) , The Foundation for the Preservation of Honey Bees, the Environmental Protection Agency as well as the contributions of beekeepers and growers who granted access to researchers.