

Report on the National Stakeholders Conference on Honey Bee Health

National Honey Bee Health Stakeholder Conference Steering Committee

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National Honey Bee Health Stakeholder Conference Steering Committee

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Disclaimer:

This is a report presenting the proceedings of a stakeholder conference organized and conducted by members of the National Honey Bee Health Stakeholder Conference Steering Committee on October 15-17, 2012 in Alexandria, VA. The views expressed in this report are those of the presenters and participants and do not necessarily represent the policies or positions of the Department of Agriculture (USDA), the Environmental Protection Agency (EPA), or the United States Government (USG).

Executive Summary

After news broke in November 2006 about Colony Collapse Disorder (CCD), a potentially new phenomenon described by sudden and widespread disappearances of adult honey bees from beehives in the U.S., the CCD Steering Committee was formed with the charge to help coordinate a federal response to address this problem. The CCD Steering Committee consists of scientists from the Department of Agriculture's (USDA) Agricultural Research Service (ARS), National Institute of Food and Agriculture (NIFA), Animal Plant Health Inspection Service (APHIS), Natural Resources Conservation Service (NRCS), Office of Pest Management Policy (OPMP), the National Agricultural Statistics Service (NASS), and also includes scientists from the Environmental Protection Agency (EPA). At that time, the Committee requested input and recommendations from a broad range of experts in apiculture about how to approach the problem. Out of this, the steering committee developed the CCD Action Plan (www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf), which outlined the main priorities for research and outreach to be conducted to characterize CCD and to develop measures to mitigate the problem. Since formation of the CCD Steering Committee early in 2007, the USDA, EPA and public and private partners have invested considerable resources to better address CCD and other major factors adversely affecting bee health.

Despite a remarkably intensive level of research effort towards understanding causes of managed honeybee colony losses in the United States, overall losses continue to be high and pose a serious threat to meeting the pollination service demands for several commercial crops. Best Management Practice (BMP) guides have been developed for multiple stakeholders, but there are numerous obstacles to widespread adoption of these practices. In addition, the needs of growers and other stakeholders must be taken into consideration before many practices can be implemented.

To address these needs, several individuals from the CCD Steering Committee, along with Pennsylvania State University, organized and convened a conference on October 15-17, 2012, in Alexandria, Virginia that brought together stakeholders with expertise in

honey bee health. Approximately 175 individuals participated, including beekeepers, scientists from industry/academia/government, representatives of conservation groups, beekeeping supply manufacturers, commodity groups, pesticide manufacturers, and government representatives from the U.S., Canada, and Europe.

A primary goal of the conference was for the CCD Steering Committee to receive input from stakeholders as they consider future actions to promote health and mitigate risks to managed honey bees in the United States. The meeting had three objectives:

1) Synthesize the current state of knowledge regarding CCD, bee pests, pathogens, and nutrition, potential pesticide effects on bees, and bee biology, genetics and breeding; 2) Facilitate the development and implementation of BMPs that stakeholders can realistically incorporate; and 3) Identify priority topics for research, education and outreach to be considered by the CCD Steering Committee for an updated Action Plan.

Dr. May Berenbaum gave the keynote address and provided an overview of the historical and current state of pollinators in the United States, from the invention of the first movable hive frame in 1852 and the first printed reference to non-target impacts of agricultural pesticides on bees in 1891, through the first U.S. detection of the parasitic *Varroa* mite in 1987 and the more recent colony declines over the past decade. Leaders in apicultural research gave comprehensive presentations of research progress on CCD, bee pests and pathogens, nutrition, pesticides, bee biology, breeding and genetics.

Highlights of Research Overviews: As noted earlier, the views expressed in this report are those of the presenters and do not necessarily represent the policies or positions of the U.S. Department of Agriculture, the Environmental Protection Agency, or the United States Government.

 Consensus is building that a complex set of stressors and pathogens is associated with CCD, and researchers are increasingly using multi-factorial approaches to studying causes of colony losses.

- The parasitic mite *Varroa destructor* remains the single most detrimental pest of honey bees, and is closely associated with overwintering colony declines.
- Multiple virus species have been associated with CCD.
- Varroa is known to cause amplified levels of viruses.
- The bacterial disease European foulbrood is being detected more often in the U.S.
 and may be linked to colony loss.
- Nutrition has a major impact on individual bee and colony longevity.
- Research indicates that gut microbes associated with honey bees play key roles in enhancement of nutrition, detoxification of chemicals, and protection against diseases.
- Acute and sublethal effects of pesticides on honey bees have been increasingly
 documented, and are a primary concern. Further tier 2 (semi-field conditions) and
 tier 3 (field conditions) research is required to establish the risks associated with
 pesticide exposure to U.S. honey bee declines in general.
- The most pressing pesticide research questions lie in determining the actual fieldrelevant pesticide exposure bees receive and the effects of pervasive exposure to multiple pesticides on bee health and productivity of whole honey bee colonies.
- Long-term cryopreservation of honey bee semen has been successfully developed and provides the means for long-term preservation of "top-tier" domestic honey bee germplasm for breeding. Genetic variation improves bee thermoregulation, disease resistance and worker productivity.
- Genomic insights from sequencing the honey bee genome are now widely used to
 understand and address major questions of breeding, parasite interactions, novel
 controls (e.g., RNAi), and management to make bees less stressed and more
 productive.

To facilitate discussion of BMPs and development of priorities, stakeholders were formed into work groups centered on the four main issues affecting bee health: 1) nutrition, 2) pesticides, 3) parasites/pathogens and 4) genetics/ biology/ breeding. The most common themes expressed in several breakout groups were:

- Federal and state partners should consider actions affecting land management to
 maximize available nutritional forage to promote and enhance good bee health and
 to protect bees by mitigating their movement into pesticide-treated crop acreage.
- Undernourished or malnourished bees appear to be more susceptible to pathogens, parasites, and other stressors, including toxins. Research is needed on forage, pollen, insect metabolic pathways, artificial and natural food sources, and food processing and storage in the hive.
- More outreach programs targeting farmers on managing potential exposure of honey bees to pesticides is needed. Efforts would benefit from involvement of beekeepers, crop consultants, pesticide manufacturers and applicators, and State lead agencies and extension agents.
- BMPs associated with bees and pesticide use, exist, but are not widely or
 systematically implemented by members of the crop producing industry. A central
 theme of the pesticides session was the need for informed and coordinated
 communication/education/extension of growers and beekeepers and the need for
 effective collaboration between stakeholders.
- Beekeepers accentuated the need for accurate and timely beekill incident reporting, monitoring, and enforcement.
- Pathogens and arthropod pests have major negative impacts on colonies.
 Management of *Varroa* and viruses was recognized as a special concern.
- Breeding emphasis is on traits, including hygienic behavior, that confer improved resistance to *Varroa* mites and diseases, such as American Foulbrood.

Although a post meeting survey was not conducted, meeting participants indicated that the conference gave them the opportunity to voice their concerns, to hear the concerns of others, and to offer their perspectives to Federal officials on future directions the government might take to ensure the future of America's pollinators. The CCD Steering Committee plans to revise the CCD Action Plan, a document that will synthesize this input. The Action Plan will outline major priorities to be addressed in the next 5-10 years. This plan will serve as a reference document for policy makers, legislators and the public and to help coordinate the federal strategy in response to honey bee losses. Finally,

given the depth of issues effecting pollinator health, consideration should be given to renaming this committee to reflect the broader range of factors discussed in this report.

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Background

In response to unexplained losses of U.S. honey bee (Apis mellifera) colonies that began to be reported in 2006 as a condition named Colony Collapse Disorder (CCD), the U.S. Department of Agriculture (USDA) established a Colony Collapse Steering Committee to lead an effort to define an approach for understanding and resolving the problem. CCD is characterized by the sudden loss of worker adults from managed hives, leading to the eventual collapse of the entire colony within a few weeks. It is a complex phenomenon, because several factors seem to be interacting to cause CCD (http://www.ars.usda.gov/is/br/ccd/ccdprogressreport2012.pdf) (CCD Progress Report 2012). The suspected factors include pests, pathogens, pesticides, nutritional deficiencies and bee hive management practices. The CCD Steering Committee, formally established in 2007, was initially composed of program leaders from ARS, NIFA, APHIS, NRCS, and NASS, the U.S. Environmental Protection Agency Office of Pesticide Programs (EPA) and two land-grant university administrators from Pennsylvania State University and Purdue University. Using input and recommendations received by university and government researchers, extension specialists and beekeepers, the steering committee developed the CCD Action Plan in July 2007 to establish key priorities for research and development of management practices to address CCD (http://www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf). Currently, the steering committee includes USDA's Office of Pest Management Policy (OPMP); formal participation of the two land-grant universities ended.

In the past five years, significant progress has been made in our understanding of the factors that are associated with CCD and the overall health of honey bees. Survey data generated by USDA (http://www.ars.usda.gov/is/pr/2011/110523.htm) indicate that overwinter losses for commercial beekeepers ranged from approximately 28 to 33 percent between 2007 and 2011 and were reported as 22 percent in 2012. It was noted in 2010-11 winter loss survey that fewer beekeepers attributed losses to CCD than in previous years (vanEngelsdorp et al. 2012), even though those reporting CCD as the cause of their losses suffered higher than average losses. Nevertheless, overall losses far exceed the historical rate (approximately 10 to 15 percent) and represent a threat to both beekeepers and to those agricultural crops that rely upon pollination as a production input. Since 2006 an estimated 10 million bee hives at an approximate current value

of \$200 each have been lost and the total replacement cost of \$2 billion dollars has been borne by the beekeepers alone (J. Frazier, unpublished).

Members of the CCD Steering Committee believed that, after five years of investigating CCD, it was necessary to assess the current state of knowledge of CCD, and of the primary factors that affect honey bee health. To this end, a subcommittee formed to plan and conduct a stakeholder conference, with the objective of seeking input from the stakeholder community regarding current understanding of research priorities, and the development of BMP's to address the needs of beekeepers and growers.

A stakeholder conference was held on October 15-17, 2012 in Alexandria, Virginia. Approximately 150 individuals were invited to the conference, including beekeepers, scientists, representatives of advocacy groups, beekeeping supply manufacturers, commodity groups, pesticide producers, academia, and State and Federal government representatives from the U.S., Canada, and Europe. The meeting was planned, organized and conducted by representatives from multiple agencies within the USDA and the U.S. EPA, along with Dr. James Frazier, Pennsylvania State University.

Conference Overview:

The goal of the conference was for officials from USDA and U.S. EPA to receive input from scientists, state governments, non-governmental organizations, industry and other stakeholders as they consider future actions to promote health and mitigate risks to North America's managed honey bees. The meeting had four aims:

• Synthesize the state of knowledge regarding CCD

Synthesize the current state of knowledge regarding each of the factors believed to be associated with declines in

- honey bee health
 - Arthropod pests and pathogens
 - Nutrition
 - o Pesticides
 - o Bee biology, genetics, and breeding

Discuss and identify priority topics for research and BMPs to be considered by the CCD
 Steering Committee for action

The first day of the meeting was devoted to examining current and recent (past 5 years) research on each of the above four factors known to affect honey bee health. Eleven researchers from land-grant universities and the USDA Agricultural Research Service (ARS) presented research summaries addressing each health-factor topic. On the second day of the conference, participants were assigned to one of four work groups in which they were encouraged to discuss viewpoints on one of the specific areas associated with honey bee health. Work group assignments were based on participants' knowledge in the topic area. Work group discussions were led by the researchers, who presented the research summaries on day one, and were facilitated by USDA and U.S. EPA personnel. The research leads, along with conference organizers, developed a set of questions designed to guide discussion within each work group (Appendix 2).

Participants reconvened during the afternoon of the second day, when recorders from each work group summarized the key questions and recommendations developed in the morning sessions. A general discussion session followed, which ensured that participants could contribute additional ideas to work groups other than to the one to which they had been assigned.

Day 1: Opening Remarks and Comments

USDA Deputy Secretary, Kathleen Merrigan, U.S. EPA Deputy Administrator, Bob Perciasepe, and USDA National Institute of Food and Agriculture (NIFA) Director Sonny Ramaswamy each provided opening remarks, addressing the importance of the issues to be discussed during the conference, and commitments by both organizations to respond to the challenges of promoting bee health while mitigating risk.

The following representatives of several stakeholder groups were also invited to provide opening comments:

- Darren Cox, Beekeeper Representative to the EPA Pesticide Program Dialogue Committee; Cox Honeyland, Logan, Utah
- Daniel Botts, Minor Crop Farmer Alliance and Florida Fruit & Vegetable Association,
 Maitland, Florida
- Dr. Gabrielle Ludwig, Senior Manager of Global, Technical and Regulatory Affairs, Almond Board of California, Modesto, California
- Dr. Barbara Glenn, Senior Vice President, Science and Regulatory Affairs, CropLife America, Washington, District of Columbia
- Laurie Davies Adams, Executive Director, North American Pollinator Protection Campaign, San Francisco, California
- Christi Heintz, Executive Director, Project Apis m., Tucson, Arizona

<u>Day 1: Research Presentations:</u> The keynote speaker was Dr. May Berenbaum of the University of Illinois Urbana-Champaign, who provided a comprehensive overview of honey bee declines. Berenbaum's presentation included an overview of historical focus on the conduct of honey bee research efforts, including challenges in experimental design and conduct yielding relevant results regarding colony health.

Leading scientists who study honey bees were identified and selected by the conference steering committee to present on a range of topics associated with honey bee health. Each presentation was followed by an open forum, during which conference participants were encouraged to ask

questions or provide commentary. Comments recorded, below, in the research summaries do not represent the expressed opinions of agencies or personnel of the USDA, the US EPA, or the U.S. Government.

Current State of Knowledge of CCD and its Relation to Honey Bee Health

(Dr. Jeff Pettis, USDA ARS, Beltsville, Maryland; Dr. Dennis vanEngelsdorp, University of Maryland, College Park, Maryland)

Summary of Research Presentation: No single silver bullet will solve the problems affecting honey bees and other pollinators. Habitat enhancement, judicious and targeted pesticide use, improved colony management techniques and improved disease and pest resistant stocks of bees are collectively needed to improve the health of honey bee colonies. It is imperative that we increase honey bee survival both to make beekeeping profitable but more importantly to meet the demands of U.S. agriculture for pollination and thus ensure of food security.

- Healthy honey bee colonies are critical for meeting the demands of food production in the United States.
- Currently, the survivorship of honey bee colonies is too low for us to be confident in our ability to meet the pollination demands of U.S. agricultural crops.
- Historically, the U.S. had as many as 6 million colonies in 1947, with declines since that time to about 4 million in 1970 and 3 million in 1990. Today's colony strength is about 2.5 million.
- Pollination demands have increased in recent years such that a single crop, almonds in California, now require over 60 percent of all managed colonies.
- Honey bee colonies have been dying at a rate of about 30 percent per year over the past few winters which leave virtually no cushion of bees for pollination.
- Because of the early almond pollination requirement, a 30 percent loss of the 2.5 million colonies would leave only 1.75 million colonies to meet the 1.5 to 1.7 million colonies currently needed in almonds. This situation leaves growers in a precarious position, and Dr. Pettis stated, "We are one poor weather event or high winter bee loss away from a pollination disaster."

 Surveys of beekeepers throughout the United States have documented this 30 percent or greater loss for five consecutive years while for the most recent winter, 2011-2012, the losses were only 22 percent.

While the lower level of loss for overwintering hives in 2011-2012 was encouraging, one year does not make a trend and reports of losses in the latter part of 2012 look like we are in for another high loss winter rate. We need to improve colony survivorship, however, honey bee health issues, including CCD, have proven to be multi-faceted and difficult to solve.

- Research into CCD and poor colony health has been unable to identify a unique causative
 agent but consensus is building that a complex set of stressors and pathogens can result in
 colony losses.
- Factors that can lead to poor health include disease and arthropod pests, pesticides, poor nutrition and beekeeping practices.
- The parasitic mite *Varroa destructor* remains the single most detrimental pest of honey bees and can magnify the role of viruses in bee health.
- Pesticide exposure to pollinators continues to be an area of research and concern, particularly the systemic pesticides such as neonicotinoids. Despite concerns regarding the potential hazard that systemic pesticides may represent to honey bee colonies, when pesticides are viewed in the aggregate at the national level, the frequency and quantity of residues of pyrethroids coupled with the toxicity of these insecticides to bees could pose a 3-fold greater hazard to the colony than the systemic neonicotinoids.
- Several studies have demonstrated that sublethal neonicotinoid exposure in immature bees resulted in an increased susceptibility to the gut pathogen *Nosema*, demonstrating that complex interaction between factors are likely contributing to poor colony health.
- Nutrition has a major impact on individual and colony longevity. There is a belief among
 beekeepers and researchers alike that land use patterns have changed to an extent where
 there is less forage available for honey bee colonies. Research is beginning to look at ways
 to diversify the agricultural landscape to increase resource availability for pollinators.
- The use of modern weed control methods in agriculture, forestry and States' Rights of Way land management have reduced availability of weeds that once provided valuable nutrition to bees.

Current State of Knowledge of Bee Biology

(Dr. Jay Evans, USDA ARS, Beltsville, Maryland)

Summary of Research Presentation: Research on honey bees involves several fields of biology, and advances in these fields are just now having an impact on maintaining healthy pollinator populations in the face of biotic and abiotic threats. New genetic and experimental approaches to address pollinator health are in use.

- A challenge to the research community is how do we weigh the relative importance of behavioral and physiological traits on bee health?
 - Understanding the relative importance of individual and 'social' traits and the tradeoffs in terms of costs of maintaining these traits, will lead to better bee breeding and management (Evans and Spivak, 2010)
 - o Pathogens and parasites of honey bees have been described in great detail, linking important microbes with negative (Runckel *et al.*, 2011; Cornman *et al.*, 2012) and positive (Anderson *et al.*, 2011; Engel, Martinson, and Moran, 2012) effects on bee health.
 - The genetics behind individual bee responses to viruses, bacteria, and gut parasites like *Nosema* (Siede, Meixner, and Büchler, 2012) and of how adult bees within the hive respond to signs of disease among their nestmates (Oxley, Spivak, and Oldroyd, 2010) are becoming more clear.
 - Evidence that infected honey bees may 'suicidally' take risks that decrease chances they will transmit disease to nestmates (Rueppell, Hayworth, and Ross, 2010) may enable more efficient breeding programs aimed at producing disease resistant bees.
- How signals shared among & between bees & their varied pests can be exploited to:
 - o Control pests, *i.e.*, by understanding how *Varroa* mites, the primary pest of honey bees worldwide, perceive vulnerable bees (Calderón *et al.*, 2009) and the means by which bees perceive and remove these mites (Harris, Danka, and Villa, 2012).
 - Manipulate foraging and other colony traits by understanding how bee behavior reflects the interplay between bee proteins, developmental stage, and environmental cues. Planned research will be extended to find key traits involved with recognizing and removing pests, such as hygienic behavior.

- Raise and maintain robust queens. For example, recent work describes how the genome of developing queen and worker bees is altered during development, revealing that a large number of genomic regions are silenced in developing bees using methylation (Foret et al., 2012), a way of 'painting' chromosomal regions into silence or activity; previously thought to be of only minor importance for insects.
- We need consistent protocols for bee research, from genetics to field experiments in order to compare data on the impacts of parasites, pathogens, nutrition and chemicals on bee health.
 - A major effort is underway to increase common practices among bee scientists and to disseminate scientific findings to the beekeeping world. The 'Beebook' (Williams et al., 2012) is a growing compendium of research protocols and insights that will enable more consistent experiments aimed at understanding bee health and bee biology. Information from the Beebook will be joined with the key venues for dispersing honey bee information among stakeholders, regulators, and researchers, including the USDA-funded Extension.org site for bee health (http://www.extension.org/bee_health), Colony Loss the network (http://www.coloss.org/) and the newly established Bee Informed Partnership (http://beeinformed.org).
- What is the current consensus on biological and abiological factors that act non-additively to impact bee health, and how do we use this knowledge?
- There is a huge shift towards multi-factorial studies in all fields of bee research. Studies of bee biology and bee health have tended to focus on one factor (one genetic trait or one environmental component) and the impacts of this factor on bee health. Recent work on non-additive interactions between chemical insults to bees and parasites (*e.g.*, Alaux *et al.*, 2010; Pettis et al., 2012), and on the interplay between nutrition and disease, exemplify the benefits of looking at problems of bee health from the standpoint of multiple inputs. Other examples include:
 - The impacts of bee genetics and the environment on bee foraging (Ament *et al.*, 2012;
 Page Jr, Fondrk, and Rueppell, 2012)

- The effects of larvae and nurse bees on the development of new queens (Linksvayer et al., 2011). This will lead to richer insights into bee biology and presumably new ideas for the management and breeding of healthy bees.
- The description of the Honey Bee Genome Project "Honeybee Genome Sequencing Consortium" (2006) has become the most-cited research paper in honey bee biology. Genomic insights are now widely used to understand and address the major questions of breeding, vetting traits, parasite interactions, novel controls (RNAi), and management to make bees less stressed and more productive.
 - o Scientists are using the power of genome-wide expression analysis to understand:
 - Bee responses to *Varroa* (Nazzi *et al.*, 2012).
 - Bee responses to poor nutrition (Alaux *et al.*, 2011).
- Results from CCD-driven studies have changed dogma related to:
 - The frequency with which bees are exposed to pathogens on flowers (Singh *et al.*, 2010).
 - o The global nature of bee parasites and pathogens (Fries, 2010).
 - o The physiological and behavioral toll of poor nutrition and exposure to chemicals (Gregorc and Ellis, 2011; Gregorc *et al.*, 2012; Henry *et al.*, 2012).
- Conclusions and Future Efforts: There remain many major knowledge gaps in bee biology, including:
 - o Impacts of nutrition and food diversity on the longevity of queens and workers.
 - o Importance and maintenance of the microbiome within the bee digestive tract.
 - Movement of parasites and pathogens across species and across continents.
 - Impacts of human barriers to spread of disease agents, including trade regulation and surveys.
 - o The causes and sustainable exploitation of what seems to be an immense genetic diversity of traits related to both individual and colony-level disease resistance.
 - The reasons behind what seem to be inconsistent, but important, bee losses due to exposure to pesticides and other chemicals.

Current State of Knowledge of Nutrition and Best Management Practices

(Dr. Gloria DeGrandi-Hoffman, USDA ARS, Tucson, Arizona; and Dr. Nancy Moran, Yale University, New Haven, Connecticut)

Summary of Research Presentation: A broad overview of recent honey bee nutrition research was presented that spanned topics from the relationship between nutrition and colony survival to the role of microbes in food processing, preservation and digestion of nutrients. Comprehensive investigation into the role of nutrition in honey bee colony health has only recently begun at the landscape, colony and molecular levels. A more in-depth understanding of the nutritional value of pollen sources and the factors affecting nutrient acquisition will provide more accurate assessments of the nutritional benefits of different pollen sources and artificial diets. We also will be able to evaluate the effects of antibiotics and pesticides on colony growth and survival from a nutritional perspective by determining their effects on nutrient acquisition and metabolism. This work will complement the need for increased bee forage and the selection of plants that would most benefit colony growth.

Specifically, the presentation included mathematical model predictions of nutrition effects on worker longevity and the repercussions on colony growth, and vulnerability to loss from parasites, such as *Varroa* mites. Information on the nutritional value of pollen and the changes in protein and amino acid concentrations after conversion of pollen to bee bread (a mixture of plant pollen, nectar, enzymes, bacteria and fungi used as food) also was provided. Recent studies have revealed new understanding about the role of nutrients and importance of beneficial microbes on honey bee health such as:

- Nutritional stress on overwintering colonies reduces the lifespan of adult workers by four days; from an average of 35 to 31 days.
- Pollen is the key protein source and bee bread is the dominant amino acid source in honey bee diets.
- Studies from several research groups reveal a distinctive set of species of gut microbes present in adult honey bees worldwide (Africa, Australia, Asia, North America, South America, Europe)
 - o Eusociality enables efficient transmission of specialized bacterial communities.

- Eight distinctive microbial species make up 95 to 99 percent of gut bacteria in most bees.
- Possible roles of microbes in bee health:
 - o Biosynthesis of needed nutrients
 - o Enzymes for pollen digestion
 - Detoxification of compounds in diet
 - Protection against parasites or diseases (*e.g.*, infection levels by *Crithidia* parasites) depends on which strains of gut bacteria are present in the bee.
- Bacterial species colonize specific regions of the adult honey bee gut.
- Two primary bacterial microbes are present in worker bees and in highly specific gut locations:
 - Snodgrassella alvi
 - o Gilliamella apicola
- Advances in genomics research are providing unprecedented opportunities to explore diversity and function of gut microbiota. Examples of studies could include the following:
 - o Bacterial sampling directly from bees or from cultured bee bacteria
 - o Massive sequencing of microbial genomic DNA
 - o Bioinformatics using databases from model bacterial systems
 - Predicting and validating functional capabilities of bacteria at the individual and colony level.
- Within a single bee, gut bacteria encode enzymes involved in the breakdown of dietary
 components, transport of sugars and amino acids, and biosynthesis of nutrients (i.e. Some
 strains of Gilliamella encode pectate lyase enzymes for breakdown of pectin, a major
 component of pollen walls).
- Antibiotic resistance genes have accumulated in bee gut bacteria in the U.S. due to history
 of frequent use for public and agricultural treatments.
- The presentation ended with specific questions that need to be addressed in future work.
 - What is next and what else do we need to know about honey bee microbiota?
 - What is the role of microbes in bee bread and honey in the hive?
 - o Do honey bee-associated bacteria help to protect against disease?
 - What is the role of microbes in making nutrients or in utilizing dietary components?

- o Can we promote beneficial microbes in the colonies by beekeeping practices?
- What are effects of antibiotic use and artificial diets on the composition and functioning of bee microbiota, both within the gut and within the colony?

Current State of Knowledge of Pathogens and Best Management Practices

(Dr. Diana Cox-Foster, Pennsylvania State University, University Park, Pennsylvania; Dr. Jay Evans, USDA ARS, Beltsville, Maryland)

Summary of Research Presentation: Greater information and knowledge about the normal microflora and pathogens associated with honey bees have been revealed through next generation sequencing and epidemiological studies and surveys. There is a dynamic ecology or flux in pathogens over time within a colony and among colonies. While new species have been discovered using metagenomics, and new pathologies have been described, including melanization (chemical defense against invasion of internal tissues by pathogens) of bee organs and brood pathologies such as "snotty brood", careful experimentation is now needed to associate novel bee health concerns with specific microbes.

• Viruses:

- New virus species have been characterized in bees in the United States.
- o Multiple virus species have been associated with CCD
- o Closely related dicistroviruses most associated with the colonies include:
 - Israeli Acute Paralysis Virus
 - Kashmir Bee Virus
 - Acute Bee Paralysis Virus
 - No detection of slow bee paralysis virus in colonies in the U.S. to date
- Most predominant viruses in U.S. are Deformed Wing Virus and Black Queen
 Cell Virus
- o Varroa is the primary factor known to cause amplified levels of some bee viruses
- o Other factors potentially affecting virus levels include:
 - Nutrition
 - Environmental chemicals (*i.e.* pesticides and in-hive miticides)

- Other pathogens
- Age of bees.
- Several questions exist concerning viruses
 - How do viruses kill bees and the colony?
 - How can viruses impact other aspects of colony health, such as behavior (learning), chemical communication, and reproduction?
 - What impact do viruses have on the brood?
- O Viral infections have also been detected in other hymenopteran pollinators and have been shown to negatively impact alfalfa leaf cutting bees and bumble bees. This raises the question whether the decline in native hymenopteran pollinators is a result of viral infections, perhaps interacting with the same stress factors affecting honey bees.

Bacterial:

- New information is available on variation among American foulbrood (*Paenibacillus larvae*, the most widespread and destructive of honey bee brood diseases) strains and the potential for these bacteria to interact with other gut microbes.
- o Some American Foulbrood strains have developed antibiotic resistance.
- European foulbrood is being detected more often and may be linked to colony loss, in contrast to its rare detection in the past years.

• Fungi:

- o Chalkbrood detected more often in colonies over the past decade.
- There are potentially other unknown fungal pathogens in bees, since characteristic symptoms are observed in some autopsies of bees from collapsing colonies.

• Microsporidia:

- Nosema ceranae widespread occurrence in U.S. colonies with some N. apis also present.
 - Data support a shift in prevalence in species composition during the last 50 years to favor *N. ceranae* over *N. apis*.
 - No widespread colony losses can be attributed to *N. ceranae* in the U.S.
 - Some colony losses may be associated with microsporidia since some beekeepers that treated with fumagillin (which kills microsporidia and other fungi) have

- reported fewer colony losses; although research data suggest that fumagillin may actually stress bees resulting in poor colony health
- New insights into N. ceranae biology and its association with bees have resulted from several cage studies.
- o Nosema genome has been sequenced.
- o *Nosema* immunosuppresses honey bees.
- O Synergism between pesticide exposure and *Nosema* infections negatively impacted bee health.
- o Nosema adversely impacts nutrient utilization.
- o *Nosema* potentially interacts with other pathogens/parasites.
- Understudied pathogens and parasites that merit more research:
 - Crithidia mellificae, a trypanosome, is highly prevalent. Adverse effects by other species of Crithidia are known in bumble bees, and it seems likely that C. mellificae has negative effects on honey bees, at least in some circumstances.
 - o Spiroplasma bacteria also occur in bees; possible seasonal effects on bee health.
 - o Both positive and negative impacts of diverse digestive tract bacteria and other microbes on bee and colony health.
- Vigilance needed to prevent introduction of pests not yet detected in U.S.
 - o Slow Bee Paralysis Virus
 - o Varroa spp.
 - New strains of Thai Sacbrood Virus

Current State of Knowledge of Arthropod Pests and Best Management Practices

(Dr. Dennis vanEngelsdorp, University of Maryland, College Park, Maryland; and Dr. Jeff Pettis, USDA ARS, Beltsville, Maryland)

Summary of Research Presentation:

- Arthropod Pests in Bees:
 - Varroa mites (*V. destructor*)
 - Recognized as the major factor underlying colony loss in the U.S. and other countries, but is not associated as a primary factor in colony collapse disorder in the United States.
 - Immunosuppresses bees and vectors viruses that infect bees.
 - Has rapidly spread into Hawaiian honey bee colonies, despite the best efforts to control its spread.
 - There is evidence for widespread resistance to the chemicals used to kill mites (miticides), *e.g.*, fluvalinate and coumaphos, and a need for development of new effective treatments and alternative methods of mite control.
 - Other treatments that beekeepers have utilized do not appear to offer effective control or may have limited use.
 - The miticide, amitraz, may provide limited control due to developing resistance in Varroa, but data indicate that the amitraz formulation is important, as the formulation used in crop-pest control has increased toxicity to bees as opposed to the formulation intended for use in-hive (ApiVar[®]).
 - The adoption of bee stocks with behavioral resistance to Varroa has not been widely utilized.
 - New insights into Varroa may result from its genome having been sequenced (Cornman, 2010).
 - Tracheal mites (*Acarapis woodi*): Not widely detected nor regarded as a major factor in U.S. colony loss.
 - Small hive beetle can lead to increased colony loss via unknown mechanisms; use of in-hive small hive beetle traps results in significantly lower colony loss.

- Africanized bees continue to spread in the U.S. and have been permanently established in several states. To help impede additional spread, an improved identification system for Africanized bees is needed along with best management practices.
- o Phorid flies are not considered to be a widespread problem or threat to colony health.
- o Bee louse and wax moths are not of major concern at the current time.
- Vigilance needed to prevent introduction of pests and other bees and wasps not yet detected in U.S.
 - o Tropilaelaps spp. (T. clareae and T. koenigerum) parasitic mite (Asia)
 - Other bee subspecies and species: A. mellifera capensis (southern Africa), A. ceranae and A. florea
 - o The Asian predatory hornet *Vespa velutina* (Asia, Europe)

Current State of Knowledge of Pesticides and Best Management Practices

(Dr. Reed Johnson, Ohio State University, Columbus, Ohio; Dr. James Frazier, Pennsylvania State University, University Park, Pennsylvania)

Summary of Research Presentation: There is broad consensus among all stakeholders that pesticide use should not affect honey bees in such a way that 1. Honey production is reduced or 2. Pollination services provided by bees are threatened (Pesticide Risk Assessment for Pollinators Executive Summary, SETAC, 2011). However, it is not clear, based on current research, whether pesticide exposure is a major factor associated with U.S. honey bee health declines in general, or specifically affects production of honey or delivery of pollination services. It is clear, however, that in some instances honey bee colonies can be severely harmed by exposure to high doses of insecticides when these compounds are used on crops, or via drift onto flowers in areas adjacent to crops that are attractive to bees.

• For example, dust produced in the process of planting pesticide-coated seeds has been shown to contain high levels of insecticide with the potential to harm bees.

- Germany 2008: Seed treatment dust containing 12 to 28 percent clothianidin or thiamethoxam (Pistorius et al., 2009, 10th Int. Symposium of the ICP-Bee Protection Group)
- U.S. 2010: Talc containing 0.3 to 1.5 percent clothianidin or thiamethoxam (Krupke *et al.*, 2012, PLoS ONE)

It is also clear, based on chemical analysis of bees and bee products, that exposure of bees to a gamut of pesticides is commonplace, but the level of exposure to any particular pesticide is generally not enough to immediately or acutely kill bees (Mullin *et al*, 2010).

- Traditional laboratory-based acute toxicity testing on adult workers (Tier 1), which determines LD₅₀ or LC₅₀ values, is required for registration of all pesticide testing.
- Acute toxicity testing does not test for effects beyond acute mortality and cannot detect
 any harm caused by pesticides that do not cause lethal effects, such as fungicides and
 herbicides.
- Acute toxicity testing cannot address sub-lethal insecticide effects on bees at levels too low to kill outright.
 - o It is relatively straightforward to determine the level of pesticides contaminating both beehives and the environment. The most pressing research questions lie in determining the true pesticide exposure that bees receive and the effect, if any, that pervasive exposure to multiple pesticides have on the health and productivity of whole honey bee colonies. Determining the actual dose of pesticide that bees receive in ecologically relevant situations will help connect laboratory-based experiments using individual bees or bee tissues to expected pesticide effects in whole colonies. How are pesticides transferred to bees and exchanged between bees?
 - O How do pesticides move within bees and the bee hive and how are these compounds metabolized and excreted by bees and bee colonies?
 - Which molecular receptors inside bees interact with pesticides?
- Can sublethal tests at the individual level predict effects on whole colonies?
 - O Drones: Sperm number and viability/Longevity
 - Workers: Foraging success/Longevity

- o Queens: Egg laying rate/Egg hatch/Longevity
- Many recent studies assessing sublethal effects in individual workers:
 - o Proboscis Extension Assay (learning) (Ciarlo *et al.*, 2012)
 - o Waggle dance behavior (Nieh et al., 2012, J. Exp. Biol)
 - o Sucrose responsiveness (Nieh *et al.*, 2012, J. Exp. Biol)
 - o Mobility (Teeters *et al.*, 2012, Env. Tox. Chem.)
 - Foraging behavior Short-term (3 hour) effects of neonicotinoids (Schneider *et al.*,
 2012, PLoS ONE)
 - o Forager loss Henry *et al.*, 2012, Science; Predict effects of forager loss on colony growth using a demographic model (Khoury *et al.*, 2011, PLoS ONE)

Laboratory tests on individual honey bees have shown that field-relevant, sublethal doses of some pesticides have effects on bee behavior and susceptibility to disease. However, it remains a challenge to measure the effects of low-level, field-relevant exposure where it matters most: in real honey bee colonies. The social complexity of honey bees and the uncontrollable aspects of field research present substantial challenges to determining pesticide effects in whole-colonies. While experiments using whole colonies have the potential to directly address the effects of pesticides on honey production and pollination services, challenges presented by field or semi-field experiments include:

- Many colonies are needed per treatment due to high variability between honey bee colonies.
- The actual levels of exposure to pesticides that bees receive are still a big question.

Computer modeling of colony demographics following pesticide exposure shows promise in linking the results of laboratory-based pesticide studies with pesticide effects on whole-colony health.

- However, model predictions depend on the parameters used (Cresswell and Thompson, 2012, Science)
- Studies in progress seek to address this concern (Zhu *et al.* 2012 unpublished); model includes analyses of:
 - o Food collection: number of foragers/food storagers
 - O Queen egg-laying: queen fitness, brood care, available cells

- o Brood: egg input, development
- Good hygiene
- o Food storage: pollen, honey

An improved understanding of the physiological basis of pesticide toxicity in honey bees could lead to an understanding of the toxicity of pesticide mixtures and the potential interactions between pesticides and pathogens, nutrition and genetics.

- Interactions to be studied:
 - o Pesticide-pesticide combinations are likely (Mullin *et al.*, 2010, PLoS ONE)
 - Average of 7.1 pesticides in pollen
 - Average of 2.5 pesticides in bees
 - Pesticide combinations can be more (or less) toxic (Johnson *et al.*, 2013, PLoS
 ONE)— need further research
 - Additive (pesticide 1 + pesticide 2)
 - o Synergistic (pesticide 1 x pesticide 2)
 - o Antagonistic (pesticide 1 pesticide 2)
 - Miticide-drug interactions Oxy-tetracycline, tau-fluvalinate (Hawthorne and Dively, 2012, PLoS ONE, multi-drug resistance transporters)
 - Many potential interactions remain to be explored: Pesticidefood/disease/season/temperature/age/genetics/management
- How can we better address the effects of pesticides on pollination and honey production?
 - Management
 - Improve communication between stakeholders
 - Further development of BMPs needed
 - Provide alternate forage
 - Continuing research being done:
 - Ecologically relevant dose
 - Modeling colonies
 - Sublethal effects
 - Interactions

Current State of Knowledge of Bee Genetics, Breeding, and Best Management Practices (Dr. Marla Spivak, University of Minnesota, St. Paul, Minnesota; and Dr. W. Steve Sheppard, Washington State University, Pullman, Washington)

Summary of Research Presentation

- Historical pattern of honey bee introductions to the New World primarily occurred between 1622 and 1922. Eight Old World subspecies were introduced, including several from Africa, the Middle East and Europe. Only three European strains found favor with U.S. beekeepers: Italian, Carniolan, and Caucasian.
- Genetic diversity is critical to honey bees colonies
 - At the intra-colony level: genetic variation improves thermoregulation, disease resistance, worker productivity, i.e., related to colony health
 - o At the population level: U.S. honey bees show effects of multiple "bottlenecks"
 - Initial introductions of limited numbers of queens, queen production methods
 (One million queens produced from less than 600 "mother" queens),
 - highly restricted importation of new breeding germplasm since 1922
- Introduction of novel Old World genetic stock for breeding
 - o USDA importation, selection and distribution of new stock (Russian Honey Bees)
 - WSU importation, selection and distribution of honey bee germplasm (semen) from original sources for three "favored" U.S. strains (Italian, Carniolan and Caucasian)
 - Recent development of practical means for long term storage (cryopreservation)
 provides means to store "top-tier" domestic honey bee germplasm for breeding use
 through "time and space" and to conserve germplasm collected from original source
 populations in Europe
- Bee Breeding Cultural Shift
 - Growing interest to produce locally, regionally adapted strains of honey bees through small scale queen production
 - Driven in part by interest to breed bees more tolerant of mites and resistant to diseases and to reduce the amount of in-hive chemical inputs (miticides, antibiotics) needed to maintain healthy bees
- Tech Transfer Teams

- Assist the bee breeding industry incorporate objective selection-trait criteria in breeding
- Help implement scientific and technological advances to enhance sustainability and profitability

• Future of Bee Breeding

- Marker-assisted selection (i.e., selection of bees that posses genetic markers for desired traits).
- Field assays for Varroa Sensitive Hygiene (VSH, a selectable trait whereby bees
 detect *Varroa* infested brood in capped cells and remove infested bee pupae,
 disrupting the mite's reproductive cycle) and other traits.
- o Increased baseline genetic diversity for trait selection.

Work Groups

On the second day of the conference, invited participants were assigned to one of four work groups and invited to address questions developed by the Steering Committee and openly discuss facts, experiences, and viewpoints on one of the areas associated with honey bee health (Appendix 3). The sections that follow contain a summary of those work group discussions. As noted earlier, the views expressed in this report are those of the presenters and do not necessarily represent the policies or positions of the U.S. Department of Agriculture, the Environmental Protection Agency or the United States Government.

Nutrition

The nutrition work group was chaired by Dr. Gloria DeGrandi-Hoffman (USDA ARS, Tucson, Arizona), and Dr. Nancy Moran (Yale University, New Haven, Connecticut). Dr. Mary Purcell-Miramontes (USDA NIFA) and Dr. Terrell Erickson (USDA NRCS) facilitated the discussion.

Questions developed to guide the discussion (Appendix 2) probed the nutritional composition of pollen before and after it is converted to bee bread and the contributions of beneficial microbes in metabolism, and food processing and storage for the hive. The discussion among stakeholders in the nutrition working group, though, primarily revolved around the shortage of high-quality forage for bees in the form of flowering plants, spatially and temporally. It was noted that availability of open foraging areas has declined drastically in the last few years, due to land use changes driven, in part, by economic and agricultural developments such as increased planting of row crops, such as corn, as commodity prices have risen, and in sites that formerly were undisturbed.

Although the purpose of this workgroup was not to recommend policies, some participants expressed the need for a land use policy that provides pesticide-free areas with blooming plants where beekeepers can safely place colonies. The primary point of this discussion was that beekeepers need access to more high-quality forage. Because of year-to-year weather fluctuations, forage areas should span a variety of regions and land types, particularly as parallels typical beekeeper migratory routes. For example, a drought in one part of the country can

drastically reduce the availability and quality of forage plants, and beekeepers need alternative sites and plants to cope with these fluctuations. Although diet supplements are essential for large-scale beekeepers, they are only a temporary substitute for high-quality floral resources. Therefore, good bee nutrition depends on how land around colonies is managed, and what flowers are available to bees.

The availability of diverse and nutritional forage was noted as being particularly important for building colony populations prior to and throughout pollination (especially of almonds) and afterward, because colonies need to recover from stresses associated with transport. Beekeepers remarked that colonies with access to good floral resources were generally healthier than those located where few floral resources exist (*i.e.* sites dominated by row crops) and fed dietary supplements. Undernourished or malnourished bees appear to be more susceptible to pathogens, parasites, and other stressors including toxins. Thus, nutrition might be a fundamental factor in mitigating negative effects of other stress factors on bee health. Issues related to Federal and State land management agencies, as well as policies or programs that affect land use and maintenance (such as The National Management Plan for Invasive Species), may be important considerations to bear on the issue of alternative forage.

It was apparent during the work group discussion that these are complex issues that will require the involvement of multiple agencies and individuals. Forage areas are impacted by various Federal and State agencies, individual landowners and growers. Management of these lands affects not only beekeepers, but also other interest groups, including environmentalists and sportsmen. There appeared to be wide agreement that solid research on the factors determining good bee nutrition will be an essential element for informing these decisions.

Questions and recommendations *generated by stakeholders* included the following:

- How much natural (or relatively unmanaged) forage is needed to support honey bees so that hives can produce surplus honey and provide vital pollination services?
- What are the benefits to agriculture (measured in increased yield) of having colonies near crops (such as soybeans and other crops not contracting for pollination services) if

- increases in yield are realized, would this affect the attitudes and practices of growers in taking steps to mitigate potential risks to bee colonies in or near their fields?
- How do particular land management practices, from right of way management to existing and potential NRCS programming, or seed mixes affect bee nutrition and movement into adjacent cropping systems?
- Farm Services Agency Conservation Reserve Program Managers: Should Conservation Reserve Program consider alternatives to expensive seed mixes currently promoted (such as prairie grass/wildflower), toward less expensive mixes with legumes, which may give growers a greater incentive? Alfalfa could serve as a cheaper way of providing pollinator habitat. Development of a cost benefit analysis related to seed mixes used on Farm Services Agency Conservation Reserve Program Lands may provide insight on possible seed mix alternatives for these scenarios.
- How do particular supplements or other bee management practices affect nutrition?
 Stakeholders stated a need to understand how bee-associated microbes play a role in the nutrition of bees, potentially enabling them to make better use of particular foods.

Pathogens and Arthropod Pests

Dr. Dennis vanEngelsdorp (University of Maryland, College Park, Maryland), Dr. Diana Cox-Foster (Pennsylvania State University, University Park, Pennsylvania), and Dr. Jay Evans (USDA ARS, Beltsville, Maryland) chaired discussion in the work group. Dr. Kevin Hackett (USDA ARS) and Dr. Robyn Rose (USDA APHIS) acted as facilitators.

There was general agreement that each question posed to guide the discussion (Appendix 2) regarding pathogens of honey bees was relevant, and further research is needed to develop solutions, but that some arthropod pests, such as small hive beetle, phorid flies, wax moth, and the bee louse, have less impact on colony health, and do not warrant increased research at this time. The group further agreed that additional information is needed about the biology of several pathogens and arthropod pests in order to develop new approaches to safeguarding honey bee health. In addition, the group recognized that new approaches to disease prevention and pathogen /arthropod pest introduction are urgently needed. Efforts toward research on disease

prevention should progress with as much synergism and coordination with international researchers and regulators as possible. These recommendations are summarized below.

Biology of Pathogen and Arthropod Pests – Research Needs:

- Develop a better understanding of interactions between honey bee symbionts, associated bee pathogens and arthropod pests.
- Pathogen and arthropod pests having major impact are in need of additional research, with Varroa being recognized of special concern, especially in association with viral diseases.
- Interaction of gut microbiome with immune systems in determining the outcome of pathogen infections needs to be better understood.
- Determine the mechanisms of pathogen and arthropod pest resistance to control tactics.
- Determine the basis for tolerance/resistance by the bees to the pathogens and arthropod pests.
- Determine the effects of different stresses (pesticides, nutrition, and climate) on disease biology in honey bees.
- Determine the role of arthropod pests in vectoring disease pathogens.

New approaches to disease prevention and pathogen/arthropod introduction

- Create a Diagnostics Decision Tree for disease diagnosis in honey bee colonies.
- Define the disease symptoms and develop a computerized diagnosis system that might be delivered as a smart phone app for use by beekeepers.
- Characterize symptoms of atypical death so it is immediately recognized and noted.
- Develop a standardized sampling method for different disease/arthropod pest symptoms
- Develop standardized diagnostics that have rapid turn-around.
- Create a centralized lab for diagnosis of samples submitted by beekeepers, researchers, and regulators.
- Develop methods to identify rogue variants or new virulent strains of pathogens/arthropod pests to allow for their rapid identification and response (see below).

Management strategies for control of pathogens and arthropods – Research/Extension Needs:

- Integrate disease surveys with surveys of management practices (*i.e.* Bee Informed survey).
- Improve integrated management tools (*e.g.*, monitoring tools) for pathogen and arthropod pest management.
- Develop new control measures for pathogens and arthropod pests, including new chemical approaches, traps, biocontrol, etc.
- Monitor for resistance in both arthropod and pathogen pests.
- Tailor approaches suitable for backyard bee keepers versus commercial operations.
- Develop novel dissemination tools (*e.g.*, smartphone apps).

Surveillance – Research/Extension Needs:

- Link and sustain different efforts that monitor bee health over time. Develop sampling methods of surveillance data to associate pest or pathogen levels with economic thresholds for bee colonies under different environmental regions of the U.S.
- Document, via surveys, as many pathogens and arthropod pests as possible, with integration of other data, such as: management strategies and control measures, nutritional state, pollen sources, crop / pesticide use in area, and climatic conditions.
- Determine what time of year works best for surveys, ideally having more than one survey/yr.
- Use the survey data to develop prediction models of bee mortality.
- Develop targeted surveys, including ports of entry with establishment of surveillance apiaries and swarm capture systems, for early detection of new arthropod and pathogen pests.
- Monitor for resistance to treatments in arthropod and pathogen pests.

Develop a rapid response network for new pathogen and arthropod pest threats

• Establish risk assessment methodologies for all known pathogen and arthropod pests.

- Create a national committee that can more rapidly respond and have the authority to carry out actions to protect honey bee colonies and the pollination industry.
- A response plan is needed that may include quarantines for newly introduced exotic pests (e.g., *Tropilaelaps*) and/or BMPs that may include destruction of infected/infested apiaries. A participant stated that it is essential that the plan include guaranteed measures for the financial compensation of beekeepers and efforts to "restock" their apiaries with 'clean' colonies.
- Synergize with similar efforts being done internationally to address bee health issues in both research and regulation arenas.

Pesticides

Dr. Reed Johnson (Ohio State University, Columbus, Ohio) chaired discussion in the pesticides work group with facilitation provided by Dr. Tom Steeger and Tom Moriarty (U.S. EPA, Washington, District of Columbia) and Terry Anderson (Consultant, ARS, Beltsville, Maryland).

A central theme throughout the work group session was the need for informed and coordinated communication/education/extension of growers and beekeepers and the need for effective collaboration between stakeholders.

(Pesticide Work Group Discussion Questions are in Appendix 2)

Best Management Practices

- Beekeepers noted that moving colonies, placed in or near crops prior to pesticide
 application can reduce the negative effects of pesticides to colonies; however, depending
 on the season, it can be difficult to move colonies of differing sizes/weights and it can be
 difficult to locate suitable places to which to move the colonies.
- Altering colony locations can result in reduced homing success by forager bees.
- Commodity group representatives noted that some growers don't require pollination services and that a knowledge gap exists between growers who need to treat pests quickly on a non-commercially pollinated crop and the potential presence of bees in the vicinity of these fields.

- Drift-WatchTM (http://www.driftwatch.org; recently renamed as FieldWatchTM), a webbased tool to help identify where and when pesticide spray operations are occurring, and the process of registering the location of beehives were discussed as ways of identifying apiary locations and promoting communication with growers. However, it was noted that in certain existing state pesticide regulation programs that do use registries (such as Drift Watch) notification (of beekeepers) is recommended 48 hours prior to application, which may not provide enough time to move colonies.
- Another concern expressed is that beekeepers on contract to one grower may not be
 protected by pesticide applications to crops in adjacent fields. Beekeepers may receive
 notification from the adjacent grower, but the beekeepers may not be able to relocate their
 colonies because of their contract to provide pollination services.
- The use of repellents to deter bees from foraging in crop areas treated with pesticides was discussed. Some research has been done in the area of repellants, but participants stated more research is needed.
- Beekeepers expressed concern about hive placement in relation to needs of sufficient water and floral sources.
 - o Supplemental diets (sugar/pollen/pollen substitutes) and supplemental water are potential means of providing uncontaminated sources of nutrition and water for bees.
 - o Beekeepers reiterated the need to develop appropriate forage areas for bees.
- Night application was identified as a potential option for growers to reduce the risk of bee exposure to pesticides.
 - Better communication/education on proper application procedures could serve as an incentive for growers to apply pesticides with short residual toxicity at night.
 - Beekeepers also are doubtful that nighttime application of fungicides is an effective risk mitigation measure for honey bees.
- Some beekeepers raised concern that fungicides don't contain pollinator language on their labels, resulting in growers and beekeepers being uninformed about potential effects on bees.
- One participant suggested that education/mitigation efforts might focus on specific crops
 that pose the greatest risk to pollinators, although identifying particular crops as
 problematic may create divisions with growers who rent land to beekeepers.

- Participants recommended providing pesticide residual toxicity (RT₂₅, the time required for 25 percent bee mortality based on the test bee population exposed to the formulated pesticide product applied to foliage) data as a label advisory to improve pollinator protection.
- "Bee safe" labeling (*i.e.*, applying a "bee friendly" logo to certain product), which has been successfully instituted in France, was mentioned as a possible incentive for industry.
- One participant noted that when EPA's registration process includes uncertainties (*e.g.* regarding potential effects), the burdens of which are unfairly born by the public. EPA therefore, should better account for potential risks before registering a pesticide. The participant also commented that EPA should have a better understanding of beekills; however, beekills often go unreported by beekeepers. (EPA is currently developing guidance to better standardize beekill investigations).

Communication/Education

- Extension information such as the Bee Informed Partnership (http://beeinformed.org/), which uses survey information collected directly from beekeepers, continue to be developed.
- There was a general sense that universities are developing materials on BMPs and pollinator protection; however, these materials may not be adequately distributed.
- A representative of Health Canada's Pest Management Regulatory Agency (PMRA)
 described the process in which apiarists are assigned to each Province to work
 collaboratively with provincial departments of agriculture, grower groups, apiarists, and
 beekeepers in Canada.
 - Crop guidance documents prepared by provinces have pollinator information that is informed by PMRA risk assessments.
 - Beekeepers contact provincial officials to report beekill incidents and to obtain additional information; however, they do not report these incidents directly to federal officials.
- Commodity group representatives in the U.S. indicated that crop advisors are reliable sources of information. Commodity group representatives also noted that:

- U.S. growers may not be aware of the affects their activities may have on bees, and stated that most growers would willingly act to protect bees, if provided the proper education.
- Commenting on the fact that relationships are often at the base of rural agriculture,
 growers report a range of experiences when working with beekeepers.
- o Many growers are unaware of potential risks associated with the newer pesticide chemistries, particularly newer compounds such as the neonicotinoids that were promoted as reduced-risk (*e.g.*., overall lower impact on human health and the environment) after passage of the Food Quality Protection Act (1996).
- Growers/applicators may not be reading labels, and their primary information for pesticide comes verbally from crop advisors, who may not be well-informed of the potential effects of newer chemistries on bees.
- It was suggested that communications between growers and their crop advisors should include information-sharing with beekeepers, current examples include:
 - o Industry organizations, such as CropLife America and Responsible Industry for a Sound Environment (RISE, an affiliate of CropLife America working in urban environments), have included sessions on pollinator issues in annual meetings with their constituents.
 - The California Department of Pesticide Regulation has initiated efforts to have pollinator protection-related materials included in Pest Control Advisor certification course work.
 - Crop advisors recognize the limited time growers may have to discuss treatment options, and so discussions and, meetings are frequently conducted at edge of field.
 Situations such as this highlight the dynamic and sometimes difficulty in consulting with beekeepers prior to pesticide application.
- Web tools were mentioned as a means of distributing regionally specific information.
- Integrated pest management centers were also identified as sources of information about alternatives to pesticides?
- Leaflets developed by the French Ministry of Agriculture to describe their registration process were discussed as an example of how certain types of management information can be distributed.

- Beekeepers suggested that they have good relationships with growers and extension agents; subscribe to various list serves to obtain crop-specific information to stay abreast of emerging treatment options they may face when their colonies are in close proximity to these crops.
- Participants indicated the need for improving the knowledge of crop advisors in bee protection practices, as well as crop protection practices.
- Webinars, blogs, list serves, social media (*e.g.*, Twitter, Facebook) and commodity-specific newsletters, especially for crops not commercially pollinated (*e.g.*, grains), were discussed as a means of distributing information.

Regulatory

- The importance of beekill reports and how they inform pesticide risk assessments was discussed.
- Accurate and timely beekill incident reporting, monitoring, and enforcement were identified as important.
 - Some beekeepers have been reluctant to report incidents for fear of damaging relationships with growers on whom they depend for pollination service contracts or honey production.
 - Concerns were expressed regarding beekeeper fear of retribution and/or distrust of government agencies they fear may cite them for illegal pesticide use for treatment of in-hive pests.
- Funding limitations have resulted in many States eliminating apiary inspectors and have also reduced extension efforts.
- Funding limitations have resulted in many States eliminating apiary inspectors and have also reduced extension efforts. This reduction in resources has led to loss of expert knowledge, thereby resulting in gaps in communication between beekeepers and growers. Stakeholders identified the need for a national coordinator that, among other things, would facilitate the dissemination of information to and between parties.

Research Needs/Funding

- Research funded by commodity groups may yield information that is not widely disseminated beyond specific, and often poorly attended, commodity group meetings.
- Beekeepers noted the need for suitable forage areas to protect bees from pesticides.
 - Minimizing bee movement from bee yards into pesticide-treated crop land with use of forage plantings preferred by bees – research into size and composition of plantings.
 - Rights-of-way management that provides beneficial pollinator habitat moves away from use of herbicides.
 - Land managers expressed concerns that invasive weed control efforts may be reducing the amount and diversity of available pollinator forage areas, particularly as associated with Rights-of-Way management.
- Stakeholders noted the need to identify "drivers" that make a difference in risk management/assessment; these may best be identified though the use of some of the forecasting models that have been developed with specific measurement end points that have the greatest effect on colony survival.
- Concern was expressed that land-grant scientists do not have incentives to engage in applied research that does not contribute to tenure/publications. Participants stated that such incentives should be increased to encourage researchers to further develop practices that mitigate the risk of bees to pesticide exposure.
- Funding mechanisms need to be identified that may allow government and university researchers to seek funds provided through industry/commodity groups without appearance of undue influence (e.g., possible use of 501c (3) organizations).
 - University researchers reported reluctance in accepting any money from industry because outside observers may question whether the resulting research was biased.
- Credibility issues were identified with industry-funded research supporting pesticide registration.
 - An opinion was expressed that the EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel selection process is biased by avoiding any associations with industry

- EPA personnel noted that under the FIFRA, the regulated community is required to provide data to support the registration of pesticides and that this burden for generating such data does not fall on taxpayers.
- Participants stated there is a need for applied research in addition to basic research to address some of the questions regarding BMP development and efficacy evaluations.

Bee Genetics, Breeding, and Biology

The bee genetics, breeding, and biology work group discussion group was chaired by Dr. Marla Spivak (University of Minnesota, St. Paul, Minnesota) and Dr. W. Steve Sheppard (Washington State University, Pullman, Washington), and facilitated by Dr. David Epstein (USDA OPMP, Washington, District of Columbia).

(Genetics/Breeding/Biology Work Group Questions in Appendix 2)

At the outset, the work group participants noted that long-term, sustainable solutions for honey bee health and productivity issues would most likely derive from selective breeding and genetic improvement of honey bees. A strong and healthy population of managed honey bees is required to provide pollination services for the agricultural sector, an activity critical to U.S. food security.

Breeding Populations: Honey bees are not native to North America, and our current managed honey bee population reflects the genetic consequences of historical introductions that included representatives from only 25 percent of the described honey bee subspecies in the Old World. Recent research indicates that North American honey bees show evidence of admixture among some of the subspecies sampled in these early introductions, a feature that may have helped U.S. honey bees avoid inbreeding-related problems (Delaney *et al.* 2009, Harpur *et al.* 2012, Sheppard 1989). As is typical with other agricultural animals and crops with Old World origins, sources of novel germplasm and genetic diversity for long-term breeding efforts can be secured through importation, quarantine, and screening of genetic material from areas of original distribution.

Breeding Tools/Trait Selection:

- Emphasis is on selection for increased hygienic behavior in commercial strains of bees.
 - Colonies expressing high levels of hygienic behavior show improved resistance to Varroa mites and diseases, such as American Foulbrood
- Work group participants indicated that a number of additional traits would be useful to
 include in breeding efforts and called for the development of both marker-assisted
 selection and improved field assays for testing various traits.
- The following traits were discussed; group consensus was that significant progress toward incorporation of these traits in breeding efforts might be expected in the short term:
 - *Varroa-Sensitive Hygiene (VSH)*
 - o *Grooming Behavior (against Varroa mite):* whereby mites are physically removed from infested adult bees, or sometimes killed by chewing (highly expressed in the species, *A ceranae*; original host of Varroa). Several labs are working on this trait; progress has been made toward identifying genetic markers that may be used to assist breeding.
 - o *Chalkbrood resistance:* Developing assays to select for resistance to this fungal disease in honey bee populations would be useful.
- Bee strains are available that express the above listed traits. However, participants recommended that association studies between traits and genetic markers be conducted, with the ultimate goal being marker-assisted selection. The rationale of this approach is that the ability to select for desirable traits within current commercial queen producer stocks would be most likely to engender acceptance among queen producers and also permit the rapid dissemination of these traits into the wider U.S. honey bee population.
- There was a general discussion of specific commercial strains of honey bees that have an innate resistance to various parasitic mites, particularly the "Russian honey bee," originating in Russia and imported by the Baton Rouge Agricultural Research Service lab. The consensus was that current commercial production of this strain (approximately 2,000 queens per year) was not likely to make a significant genetic impact on overall commercial production of queens in the United States (approximately 1 million queens annually). However, specific traits associated with this strain (mite tolerance) and with USDA-developed VSH bees (mite resistance) are highly desirable and an effort should be

- made to select for or otherwise incorporate similar traits within the U.S. breeding population.
- Germplasm Repository: The recent development and improvement of cryopreservation methods for honey bee semen provides significant opportunities for honey bee breeders. Workshop participants discussed the potential importance of establishing a honey bee germplasm repository and supporting research on honey bee cryopreservation to evaluate storage characteristics and limitations. Cryogenic reproductive technology is widely used in breeding programs with a number of agricultural animals (e.g., turkeys, sheep, beef and dairy cattle, and swine) and has been responsible for significant improvements in measures of stock productivity where it has been introduced. Cryogenic preservation of bee germplasm resources provides both a means to address conservation needs and practical breeding goals.
- A honey bee germplasm repository would serve as a place to maintain (for many years or decades) novel honey bee germplasm of three subspecies (*A. mellifera ligustica*, *A. mellifera carnica*, and *A. mellifera caucasica*) currently being imported from the Old World under a permit granted by the Animal and Plant Health Inspection Service.
 - At present, aliquots of this semen are being used to inseminate U.S. queen bees for release in a collaborative project with western U.S. queen producers and others are maintained in cryogenic storage as part of a long-term breeding program.
- A germplasm repository would allow for the preservation of "top tier" domestic genetic resources from the current U.S. honey bee population.
 - Queen producers could cryopreserve examples of their best lines of honey bees and then, years or decades later, retrieve these from liquid nitrogen storage to backcross to extant populations.
 - Such a repository effectively provides the option for queen producers to breed across time (different year classes) and space (easy transportation of genetic material) in ways previously unavailable.
- Technology Transfer Teams: Workshop participants also discussed the establishment and support of Tech Teams regionally within the U.S. to assist beekeepers. The system discussed was based on a model in use in Canada and another one currently operating in

California to assist queen breeders. The concept of the Tech Team is that a group of trained individuals work in the field with beekeepers to assess stocks and provide information that would inform management decisions to assess and breed bees (in the case of queen producers) or maintain colony health (all locations). The approach represents a new, field-active model of extension and a tool for action at the interface of science (applied research) and industry (informed management). The model calls for a fee-for-service approach that will make the tech teams self-supporting within a few years.

- The Tech Team currently assisting California queen bee breeders provides data that allows producers to assess their genetic stocks for specific traits of interest (hygienic behavior, for example). The teams are in place to also provide selection assistance to breeders as other traits become available. The Tech Team approach provides a means to incorporate objective criteria into the breeder's traditional process of choosing breeding queens.
- In addition to assisting the industry with the implementation of research findings such as genetic improvement (in California) or colony health (California, and Midwestern and Southeastern states), Tech Teams also provide a means for capturing data on current honey bee populations that can be used for epidemiological analyses or breeding (through identification of high-quality stocks).
- A new Tech Team is starting up in the Midwest as part of the Bee Informed Partnership (http://beeinformed.org/), and there is strong interest to develop a Tech Team for the southeastern United States.
- Diagnostic Laboratories: Few diagnostic laboratories are available in the United States to support beekeepers that wish to submit samples of their bees for determination of pathogen and parasite loads. Work group participants discussed the utility of establishing one or more diagnostic laboratories tasked with providing rapid turnaround analyses of pathogens and parasites for Tech Teams and beekeepers.
- The primary organisms that need to be analyzed by diagnostic laboratories include *Nosema spp.*, *V. destructor*, and tracheal mites (*Acarapis woodi*), although these laboratories could also be useful in evaluating submitted stocks for genetic markers for trait selection, as that technology becomes available.
- In areas where Africanized honey bees occur, there would also be a demand to analyze samples to determine the extent of genetic introgression from Africanized honey bees.

- Other Issues: The following additional topics were noted by participants as needing additional research:
- Queen failures: There is a widespread perception that honey bee queens do not live as long as they used to. Research into the possible causes of early supersedure, the process by which one queen bee is replaced by a new queen, or queen failure without replacement is needed to determine prevalence and causes, such as genetics, pathogen, pesticides, nutrition, management, and shipping.
- Genetically based treatments for pathogens: Interference RNA (RNAi), technology research is needed on honey bees and other pollinators. RNAi is a process used by many different organisms to regulate the activity of genes, and is also known as post-transcriptional gene silencing.
- Signaling and communication: Basic research is needed to understand signaling and communication within the colony and between pests and bees.
- Lack of research funding for applied bee issues: Work group participants also discussed the difficulty that researchers have in finding adequate funding to carry out studies in applied bee research. Participants recommended exploring whether USDA could develop a call for proposals on applied issues in colony health and beekeeping sustainability.

Conclusion

Overall and consistent with the stated objective, this conference provided an overview of a significant body of new knowledge on the current state of the science of honey bee health to the CCD Steering Committee that will be helpful in updating the CCD action plan. Stakeholders also identified a number of BMPs to potentially address factors associated with declines, and research needs were clearly articulated as well toward addressing uncertainties. In response to stakeholder input provided at the conference and based on the available science and its associated uncertainties, the CCD Sterring Committee will revise the CCD action plan, The purpose of the action plan is to synthesize current recommendations from stakeholders and to coordinate an updated Federal strategy to address honey bee losses. The decline of honey bees and other pollinators continues to be a high priority topic for the USDA and the U.S. EPA. Intramural and extramural research and extension to elucidate the factors associated with losses and mitigating risks remains a high priority. We anticipate that the next CCD action plan will be completed in 2013 to early 2014.

Acknowledgment

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Appendix 1. Conference Agenda

National Stakeholders Conference on Honey Bee Health

October 15-17, 2012

Sheraton Suites Old Town Alexandria, 801 North St. Asaph Street, Alexandria, Virginia

Agenda: Day 1, October 15, 2012

Plenary session

8:00AM – 8:30AM: Opening Remarks: USDA Deputy Secretary, Kathleen Merrigan; U.S. EPA Deputy Administrator, Bob Perciasepe.

8:30AM – 8:40AM: Welcome, Sonny Ramaswamy, USDA NIFA Director

8:40AM – 9:30AM: Keynote Speaker, May Berenbaum, University of Illinois, Urbana-Champaign: Overview of the State of Our Pollinators

9:30AM-10:40AM: Stakeholder Opening Comments

Presenters: Darren Cox, Beekeeper Representative to US EPA Pollinator Program Dialogue Committee; Dan Botts, Minor Crop Farm Alliance; Gabrielle Ludwig, Almond Board of California; Barbara Glenn, Senior VP, Science and Regulatory Affairs, CropLife America; Laurie Davies Adams, Executive Director, North American Pollinator Protection Campaign; Christi Heintz, Apis m

10:40AM – 11:00AM: Break

Topic Presentations:

11:00AM – 11:30AM: Current State of Knowledge of CCD and its Relation to Honey Bee Health; Jeff Pettis, USDA ARS; Dennis van Engelsdorp, University of Maryland

11:30AM – 12:00AM: Current State of Knowledge of Bee Biology; Jay Evans, USDA ARS

12:00PM – 1:30PM: Lunch

1:30AM – 2:00PM: Current State of Knowledge of Nutrition and Best Management Practices; Gloria DeGrandi-Hoffman, USDA ARS, Tucson, Arizona; Nancy Moran, Yale University

2:00PM – 2:30PM: Current State of Knowledge of Pathogens and Best Management Practices; Diana Cox-Foster, Pennsylvania State University; Jay Evans, USDA ARS

2:30PM – 3:00PM: Current State of Knowledge of Arthropod Pests and Best Management Practices; Dennis vanEngelsdorp, University of Maryland; Jeff Pettis, USDA ARS

3:00PM – 3:30PM: Break

3:30PM – 4:00PM: State of Knowledge of Pesticides and Best Management Practices; Reed Johnson, Ohio State University; Jim Frazier, Pennsylvania State University

4:00PM – 4:30PM: Current State of Knowledge of Bee Genetics, Breeding and Best Management Practices; Marla Spivak, University of Minnesota; Steve Sheppard, Washington State University

4:30PM – 5:00PM: Break

5:00PM – 7:00PM: Evening Discussion/Networking Session – Transition to Day 2 Work Group Sessions

Day 2, October 16, 2012

8:00AM – 10:00AM: Work Group Sessions

- Nutrition

- Pathogens and Arthropod Pests

- Pesticides

- Bee Genetics, Breeding, Biology

10:00AM – 10:20AM: Break

10:20AM – 12:00PM: Work Group Sessions

- Nutrition

- Pathogens and Arthropod Pests

- Pesticides

- Bee Genetics, Breeding and Biology

12:00PM -1:30PM: Lunch

1:30PM – 3:10PM: Conference participants reconvene in general session

- Work Group Reports to the Whole:

1:30 - 2:15: Nutrition

2:15 - 3:00: Pests

3:00PM – 3:20PM: Break

3:20PM – 5:00PM: Conference participants reconvene in general session

- Work Group Reports to the Whole:

3:25 – 4:10: Bee Genetics, Breeding, Biology

4:10 - 5:00: Pesticides

Day 3, October 17, 2012

8:30AM – 10:00PM: Federal CCD Steering Committee meeting with research leaders to summarize conference input.

10:00 AM – 12:00PM: Federal CCD Steering Committee meeting to revise Federal CCD Action Plan.

Conference Steering Committee:

David Epstein, USDA OPMP; Tom Moriarty and Tom Steeger, US EPA; Kevin Hackett, USDA ARS; Robyn Rose, USDA APHIS; Mary Purcell-Miramontes, USDA NIFA; Terrell Erickson, USDA NRCS.

Appendix 2. Questions Developed for Day 2 Work Groups.

Questions developed for discussion in the Nutrition Work Group:

- 1) How do we evaluate the nutritional value of pollen? How does the nutritional composition of pollen change after it is converted to bee bread?
- 2) How do protein and carbohydrate supplements affect beneficial gut microbes?
- 3) Does pollen and nectar contamination with pesticides/fungicides affect beneficial microbes in stored pollen and the bee's digestive system?
- 4) Is there an interaction between nutritional status in a colony and its susceptibility to disease and parasites? Is a colony's response to treatments for foulbrood, Nosema or mites affected by nutrition?
- 5) How can we balance treatments so that beneficial microbes are not negatively affected, while still controlling pathogens and pests?
- 6) Has anyone noticed apparent detrimental effects from treatments with Tylosin®, Terramycin®, or other anti-microbials?
- 7) What research projects would add most information to understanding how microbes in colonies are affecting colony health?

Questions developed for discussion in the Pathogens Work Group:

- 1) What are the best ways to describe a pathogen/disease so that others can determine if they have the same organism?
- 2) What are the health impacts of 'neglected' parasites/pathogens/or potential symbionts like Crithidia, fungi, amoebae, lactobacteria, spiroplasma?
- 3) How best to sample, preserve and screen samples for disease both unknown and known?
- 4) How can surveys be better used to predict/mitigate disease (targets, time scales, costs)?
- 5) Which management processes are responsible for enabling disease spread/minimizing disease occurrence?
- 6) Are other pollinator species also being impacted by viral infections and common stress factors?

- 7) How does Varroa increase virulence of transmitted viruses via impacts on bee immunity and impacts on viral load and 'readiness'?
- 8) Can a single method be developed to sample adult bees or brood that will work for most pests and pathogens or do we need specific sampling regimes for each?

Questions developed for discussion in the arthropod pests Work Group:

- 1) Can Varroa and European honey bees reach stable host-parasite equilibrium if we reduce chemical controls?
- a. Do we have commercial stocks that are viable for pollination?
- b. Are chemical treatments doing more harm than good?
- 2) Rank the following in terms of importance for dealing with Varroa new chemical controls, new biological controls, understanding of resistance mechanisms by Africanized and Asian honey bees, means to understand and disrupt the mite-virus interaction.
- 3) Are the current traps and chemical controls adequate for dealing with small hive beetle and if not what are areas of research that would be most helpful?
- 4) Have chemical treatments for Varroa made tracheal mites scarce or has natural selection driven tracheal mite levels down?
- 5) Are tracheal mites still an issue in bee health?
- 6) Should research be directed at novel or rare pests (*i.e.* phorid flies, the bee louse Braula, etc)?
- 7) Should research be conducted on known threats from abroad and if so rank the following?

 (Tropilaelaps mites, Apis cerana, capensis honey bees and Thai sacbrood)
- 8) What do we need to know about the lifecycles of honey bee threats not found in the U.S. in order to be prepared for eradication efforts and/or providing management advice in the event of their introduction?
- 9) Do we need new control methods for wax moths?
- 10) How best management practices are effectively disseminated through the beekeeping community?

- 1) Best Management Practices:
- a. Are there sources for grower/beekeeper BMPs that are currently in use (Project *Apis m*)?
- b. What practices do <u>beekeepers</u> use or prefer in order to minimize the potential impact of pesticides to managed honey bees? Do practices differ by crop or region?
- c. To what extent are growers aware of the potential impact their activity may have on bees?
- d. What practices do <u>applicators or growers</u> use or prefer to minimize the potential impact of pesticides to managed honey bees?
- 2) Who/what are the best information sources for growers when choosing products to protect crops?
- a. Do these sources (such as Pest Control Advisors (PCAs) have access to information on best management practices with respect to pollinator protection?
- b. Do beekeepers consult with these sources (such as PCAs) or with growers to work out management practices that may present lower potential risk from pesticides? If not, why not?
- 3) What options are available to improve communication between stakeholders (state officials, growers, applicators and beekeepers improve risk management?
- 4) How can stakeholders (state officials, beekeepers, growers, and applicators) work together to build integrated plans to protect against pests insects and protect managed pollinators?
- 5) Are there efforts underway to develop Best Management Practices that apply to pesticide use in agricultural settings? Are there efforts underway to develop Best Management Practices that apply to in-hive use of pesticides? If so, what is likely to result from these efforts? If not, why not? Are there exposure scenarios or routes that stakeholders feel have not been identified by federal/state regulatory partners?
- 6) Does reserve/non-crop land provide a pesticide-free forage scenario for managed bees, and if not, why and how can it be managed?
- 7) How can a beekeeper know if pesticides exposure is a factor in colony loss or weakening?
- a. At the colony level, how does acute exposure to a pesticide differ from that of chronic exposure?

- 8) Historically, what is the typical loss a beekeeper experiences from pesticides?
- a. Aside from obvious losses, do beekeepers feel that delivery of pollination services and honey production have been affected by pesticide exposure?
- b. Do beekeepers feel that current loss due to pesticides is equally associated across crops, or across the country? That is, do beekeepers feel that their losses from pesticides would be different if they worked in different states or contracted with different crops?
- 9) Do miticides cause losses? What is an acceptable level of loss due miticide exposure? Can be ekeeping survive and be profitable without use of miticides?
- 10) To what extent are alternative forage areas a viable option in heavily developed agricultural areas?

Questions developed for discussion in the Pesticide Work Group: Research

- 11) Can we directly measure the effects of pesticide exposure on delivery of pollination services and honey production? Can other, more easily measured, endpoints be used to predict pesticide effects on pollination and honey production? Is colony strength an adequate measurement endpoint?
- 12) Can sublethal pesticide exposure be shown to affect pollination and honey production? How can we relate sublethal exposure effects of individual bees (PER, mobility, homing) to whole colony success? Can sublethal testing on individuals be improved?
- 13) How can we get to a better estimate of bees' pesticide exposure? Is it possible to estimate individual bees' body burden (the Ecologically Relevant Concentration) of pesticide through empirical measurement or toxicokinetic/toxicodynamic modeling?
- 14) Interactions could occur between insecticides, miticides, fungicides, herbicides, adjuvants, pests, pathogens, nutritional status, microbial community, plant xenobiotics, seasonality, management practices, caste, life stage and genetics. Some combinations are likely to be either harmful or beneficial to bees how do we discover these without testing all possible combinations?
- 15) How do we pay for pesticide-related bee research?

Questions developed for discussion in the Genetics/Breeding/Biology Work Group:

- 1. Genetic Diversity: Genetic diversity of the honey bee may now be considered on a global scale. For example: the total diversity of managed "Italian" honey bees may be best represented by honey bees from Italy (the original subspecies) and managed populations in the Americas and Australia. All of these may be viable pools that could contribute to establishing populations for selective breeding. A cryogenic storage facility could maintain germplasm from both natural and managed honey bee populations for future breeding. Thus, in addition to Old World source populations, genetic samples of specific desirable commercial lines of bees could be placed into cryogenic storage for later recovery. Cryogenic storage addresses an overarching USDA mandate to preserve germplasm from animals and plants of agricultural significance: "The mission of the National Center for Genetic Resources Preservation (NCGRP) is to acquire, evaluate, preserve, and provide a national collection of genetic resources to secure the biological diversity that underpins a sustainable U.S. agricultural economy through diligent stewardship, research, and communication." Despite initial efforts to sustain a honey bee stock center, the cost needed to maintain genetic diversity in large cohorts of living colonies was prohibitive. Now that functional cryopreservation technology is available, is it time to reconsider the status quo? That is: Is there a need for a major effort to establish a national honey bee germplasm repository?
- 2. Breeding Commercial bee breeding: The goal of the tech-transfer "Bee Team", funded by the Bee Informed Partnership and fees-for-service, is to work directly with bee breeders in California to improve stock selection, enhance genetic diversity, and engage in disease and parasite-related diagnostic evaluations. In addition to helping bee breeders keep track of and select colonies with the lowest mite, Nosema and virus levels, the Bee Team assists with selection for hygienic behavior using the freeze-killed brood assay.
- a. What other traits could be selected? Are we ready to implement marker-assisted selection for grooming behavior and VSH (e.g., the Bee Team could send samples to a lab for genetic testing)?

- b. Toward a sustainable and diverse genetic base: What is the best way to incorporate additional imported honey bee genetic material into the actual breeding populations of the U.S.?
- Local/regional bee breeding: Many beekeepers would like to select for one or more of the following: "locally adapted" stock; survival stock; and/or stock that does not require any chemical treatments.
- a. How to balance genetic diversity and selecting for resistance, while trying to keep things locally adapted? What does "locally adapted" mean in terms of honey bees?
- b. Given the perceived differences in selection criteria between large commercial interests and beekeepers working to develop locally selected populations, how do the roles of subspecies origin, selection criteria for pest and parasite control and overwintering strategies inform the choice of the initial population for breeding?
- 3. Queen Failures: Real or Perceived?
- a. If real: Is this problem tied to race, stock, type of beekeeping operation, old vs. new combs? Is the problem caused by not enough time spent in mating nucs? Or pathogen (viruses, Nosema?) Or pesticide residue?
- 4. How do we weigh the impacts of behavioral and physiological (immunity, development) traits on bee health? Similarly for individual and 'social' traits? There must be trade-offs for bees, in terms of costs of maintaining these traits, so we can't just push them to be above average at everything.
- 5. How can bee x bee and bee x pest signaling be exploited to 1) control pests, 2) manipulate foraging and other colony traits, 3) maintain respect for the queen?
- 6. How can standards and protocols be normalized across labs and countries: controlled language, Beebook for protocols, true Standard Operating Procedures? These are all needed, especially with touchy regulatory issues.
- 7. What is the current consensus on biological and abiological factors that act non-additively to impact bee health, do any cancel each other out or is it always 1 + 1 ≥ 2? How do we use this knowledge (e.g., are survey tools economic for making management decisions? Can knowing that certain factors interact negatively for bees be used to more strongly regulate those factors when they are likely to co-occur?

- 8. Six years post-honeybee genome what have we learned about bee biology and what is in place for the major questions of breeding, vetting traits, parasite interactions, novel controls (RNA*i*), and management to make bees less stressed and more productive.
- 9. Six years post-CCD what have we learned from the added efforts put into bee disease and responses to chemical stresses? Which new tools or ways of thinking/models are going to change the field and improve bee health?
- 10. Will the world accept genetic strategies from i) RNAi versus pests, ii) RNAi to influence bee behaviors? iii) germline transformation of bees even if it tackles a critical weak point like viral resistance?

Appendix 3: Invited Work Group Participants

(The participant lists in this appendix do not represent all who actually participated in each work group. Some invitees did not attend the conference. Note that recorders are not listed.)

Invited Work Group Participants - Nutrition

Leaders

Degrandi-Hoffman, Gloria USDA ARS

Moran, Nancy, Yale University

Purcell Miramontes, Mary USDA NIFA

Participants

Erickson, Terrell,

Berger, Lori California Specialty Crop Association

Browning, Zach Browning Honey Co. Inc.

Davies-Adams, Laurie North American Pollinator Protection Campaign

USDA NRCS

Delaney, Deborah University of Delaware

Downey, Danielle Apiary Inspectors of America

Hayes, Jerry Monsanto/Beeologics

Heintz, Christi Project Apis m

Johnson, Jody Smithers Viscient

Kelly, Iain Bayer CropScience

Kuivila, Kathryn USGS

Ludwig, Gabrielle Almond Board of California

Overmyer, Jay Syngenta Crop Protection

Verhoek, Randy Harvest Honey Inc ND, NHBA

Wehling, Wayne USDA APHIS

Unable to Attend

Eischen, Frank USDA ARS

Esaias, Wayne UMD/NASA (emeritus)

Haun, Gray Tennessee Department of Agriculture

Hyberg, Skip USDA FSA

Mussen, Eric California Department of Food and Agriculture

Rao, Sujaya Oregon State University

Sanroma, Joe LA Beekeepers Association, AHPA

Trumble, John UC Riverside

Tucker, Tim Tucker Bees, Kansas, ABA

Vaughn, Mace Xerces Society

Wardel, Gordy Paramount Farms, CA

Invited Work Group Participants – Pathogens/Arthropods

Leaders

Cox-Foster, Diana Pennsylvania State University

Evans, Jay USDA ARS Hackett, Kevin USDA ARS

Rose, Robyn USDA APHIS

van Engelsdorp, Dennis University of Maryland

Participants

Abbott, John Syngenta Crop Protection

Burand, John University of Massachusetts

Caron, Dewey University of Delaware

Cox, Darren Cox Honeyland, NHBA

Coy, Steve Coy's Honey Farm

Cruise, Chris Mann Lake Beekeeping Supplies

Feken, Max Florida Dept of Agriculture and Consumer Services

Glenn, Barb CropLife America

Hackenberg, David Hackenberg Apiaries, ABF

Huang, Wei-Fone University of Illinois

James, Rosalind USDA ARS
Meikle, William USDA ARS

Rogers, Dick Bayer CropScience

Skinner, John University of Tennessee

Smallwood, Ben USDA NRCS

Stewart, Colin USDA APHIS

Stoner, Kimberly Connecticut Agricultural Experiment Station

Teal, Peter USDA ARS

Webster, Tom Kentucky State University

Westervelt, David Florida Dept of Agriculture and Consumer Services

Unable to Attend

Averill, Anne University of Massachusetts

DiSalvo, Carol National Park Service

Haterius, Stephen National Association of State Departments of Agriculture

Kozak, Paul Canadian Association of Professional Apiculturists

Kramer, Vince Dow AgroSciences

Levi, Ed Arkansas State Plant Board

Invited Work Group Participants - Pesticides

Leaders

Johnson, Reed, Research Leader, Ohio State University

Steeger, Tom, Facilitator, U.S. EPA Office of Pesticide Programs

Moriarty, Tom, Facilitator, U.S. EPA Office of Pesticide Programs

Anderson, Terry Facilitator, USDA ARS

Participants

Adee, Bret American Honey Producers Association

Alix, Anne Dow AgroSciences

Berenbaum, May University of Illinois, Champaign-Urbana

Bireley, Richard California Dept. of Pesticide Regulation

Christiansen, Jessica Monsanto/Beeologics

Egan, Peter Armed Forces Pest Management Board

Fischer, David Bayer Crop Science

Hansen, George American Beekeepers' Federation

Harriot, Nichelle Beyond Pesticides

Hart, Connie Canada Pest Management Regulatory Agency

Hooven, Louisa Oregon State University

Hou, Wayne Canada Pest Management Regulatory Agency

Johansen, Erik Washington State Department of Agriculture

McCain, Pat Syngenta Crop Science

Mendes, Dave Commercial Beekeeper, FL

O'Neill, Bridget DuPont Chemical

Parker, Don National Cotton Council

Pettis, Jeff USDA ARS

Ruckert, Ed McDermott Will & Emery LLP

Seetin, Mark U.S. Apple Committee

Tindal, Nick Association of Equipment Manufacturers

Trainer, Maria CropLife Canada

Walker, Larissa Center for Food Safety

Wisk, Joe BASF

Unable to Attend

Dively, Galen University of Maryland

Lu, Chensheng Harvard University

Rowe, Brian Michigan Department of Agriculture

Sass, Jennifer Natural Resources Defense Council

Tignor, Keith Apiary Inspectors of America

Willet, Mike Northwest Horticultural Council

Wu, Mae Natural Resources Defense Council

<u>Invited Work Group Participants – Biology/Genetics/Breeding</u>

Leaders

Epstein, David USDA ARS

Sheppard, Steve Washington State University

Spivak, Marla University of Minnesota

Participants

Bobb, Jim Eastern Apiculture Society

Boess, Bruce USDA NASS

Culiney, Tom USDA APHIS

Danka, Robert USDA ARS

de Guzman, Lilia USDA APHIS

Dolezal, Adam Iowa State University

Dykes, Mark Florida Dept of Agriculture and Consumer Services

Hawthorne, David University of Maryland

Henderson, Colin Bee Alert Technology

Mattila, Heather Wellesley College

McCallister, Ray CropLife America

Pruisner, Robin Iowa Dept of Agriculture

Rinderer, Tom USDA ARS

Rouse, Gus Kona Queen Hawaii, Inc

Rueppell, Olav University of North Carolina

Tarpy, Dave North Carolina State University

Zisook, Elsa Valent U.S.A. Corporation

Unable to Attend

Bourgeois, Lanie USDA ARS

Buchman, Steve Pollinator Partnership

Cane, Jim USDA ARS

Derisi, Joe University of California San Francisco

Sagili, Ramesh Oregon State University
Weaver, Danny BeeWeaver Apiaries, TX

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