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USDA Varroa Mite Summit

February 18-19, 2014

Summit Held at the USDA

Animal and Plant Health Inspection Service

Riverdale, MD



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2014 USDA *Varroa* Summit 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 USDA *Varroa* Summit Conference Steering Committee on February 18-19, 2014 in Riverdale, MD. The views expressed in this report are those of the presenters and participants and do not necessarily represent the policies or positions of the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA), or the United States Government (USG).

Conference Overview

The goal of the conference was to enable administrators from the USDA and US EPA to receive input from scientists, state governments, non-governmental organizations, industry, and beekeepers on the state of current research as well as obtain recommendations of future goals to minimize detrimental effects of *Varroa destructor* on honey bee health.

Opening Remarks and Comments

The individuals listed below provided opening remarks addressing the importance of the Varroa mite's detrimental effect on honey bee health, and the immediate need to respond with methods and tools to mitigate the numerous deleterious effects the mite has on bee health.

Speakers and Representatives

- Opening Remarks/Welcome
 - Jeff Pettis, Research Leader, USDA-Agricultural Research Service (ARS)
 - Ann Bartuska, Deputy Under Secretary, USDA Research, Education and Economics (REE)
 - Jim Jones, Assistant Administrator, US Environmental Protection Agency (USEPA)
 - Barbara Glenn, CropLife America
 - Gabriele Ludwig and Bob Curtis, California Almond Board
 - Brett Adey, Past President, American Honey bee Producers Association
 - Tim Tucker, President, American Bee Federation

Executive Summary

The 2013 “*Report on the National Stakeholders Conference on Honey Bee Health*” (<http://www.usda.gov/documents/ReportHoneyBeeHealth.pdf>) highlighted the parasitic mite, *Varroa destructor*, as a primary factor affecting the health of European honey bee, *Apis mellifera* L. (hereafter referred to as honey bees and or *A. mellifera*), populations, stating that the Varroa mite is “... *the single most detrimental pest of honey bees, and is closely associated with overwintering colony declines.*” Following this report and at the request of the American Honey Producers Association and the National Cotton Council, the Colony Collapse Disorder (CCD) Steering Committee held a summit to focus on current research related to Varroa, and the challenges and future goals in managing *Varroa*. The *Varroa* Summit was convened on February 18-19, 2014 at the USDA Animal and Plant Health Inspection Service (APHIS) facility in Riverdale, MD. A primary goal of the summit was for the USDA to receive input from stakeholders and bee researchers to help guide future actions to promote health and mitigate risks to managed honey bees from Varroa mites in the United States. The meeting had three objectives: 1) Synthesize the current state of knowledge regarding *Varroa* management; 2) Identify priority topics for research, education and outreach, and 3) Encourage and facilitate the development and implementation of best management practices (BMPs) that stakeholders can realistically incorporate.

Approximately 75 invited guests participated in the summit; participants included beekeepers, agricultural commodity group representatives, researchers, and representatives of the crop protection industry and federal and state agencies. The 2-day summit featured plenary sessions in which research by federal, industry and university scientists from the United States and Europe was highlighted. Four smaller workgroup sessions provided input on key priorities for future research. The key priority areas discussed in the smaller workgroup sessions included: 1) *Varroa* biology/effects on colony survival; 2) breeding resistant bees; 3) mite control options/ resistance management; and, 4) interaction of *Varroa* with pathogens and nutrition.

Participants identified several key knowledge gaps in understanding *Varroa*. There is little known about *Varroa destructor's* parasitism on its natural host, *i.e.*, the Asian honey bee (*Apis cerana*). *Varroa* appears to do little damage to its natural host, so understanding this host-parasite relationship may be fundamental to understanding *Varroa* management on the European honey bee, *Apis mellifera*. Once the mite's life cycle and its mechanisms for transmitting disease, *i.e.*, viruses, are identified in its natural host, it may be possible to disrupt its life cycle and viral transmission in *A. mellifera*. We know that high mite levels exacerbate viral problems, but the mechanism for this interaction is unknown. In the short term, breeding for bee resistance (*e.g.*, *Varroa* sensitive hygienic behaviors (VSH)) and/or the viruses it vectors can help to minimize the destructive effects of the mite in *A. mellifera*. . Through genome comparison of *A. cerana* and *A. mellifera*, it will likely be possible to create better virus-resistant honey bee lines.

Together, with improved management strategies, the participants agreed that accessibility of adequate tools for monitoring is an important issue for commercial and small scale beekeepers. Development of tools that would accurately measure and monitor mite populations, as well as easy to use field diagnostics (*e.g.*, metrics such as healthy brood patterns that indicate honey bee hive health) were stressed as immediate priorities. Additionally a gap that can be addressed in the short-term is improvement of outreach programs to beekeeping communities to assist in monitoring efforts using existing tools.

Among all participants there was a strong consensus that *Varroa's* effects on *A. mellifera* need to be researched from a molecular standpoint. Major short and long term research goals identified in work group sessions included: 1) identification of *Varroa* and or virus resistant or tolerant genes in *A. mellifera*, 2) identification of genes associated with avirulent mites and mites with low reproductive capacity (fecundity), 3) development of new chemical and non-chemical *Varroa* control measures, , and biotechnological methods and tools to disrupt mite reproduction, such as RNAi, 4) identification of resistance mechanisms to *Varroa* in original host bees, *A. cerana (i.e.*,

use of basic life history studies and molecular mechanisms to better understand the mite's life cycle and vulnerabilities), 5) establishment of a diagnostic laboratory with capabilities that meet world standards for trade similar to those in the European Union and Canada, and 6) improvement and harmonization of BMPs.

The Bee Informed Partnership (BIP) "Tech Teams" were highlighted as an effective means of communicating. These teams work directly with beekeepers to evaluate the condition of their hives and to communicate research findings to them. Participants agreed that there is a need for a National Extension Agent and a single, reliable website dealing with bee health to accurately transfer knowledge from scientists to both the bee industry and commodity groups that depend on bees for pollination. Many of the topics outlined above have some level of research or extension effort underway but lack the resources to be fully realized.

Research Presentations

Summary

The consistent theme of the presentations was the need for development of new methodologies to reduce mite transmission, while mitigating the risk to the colonies that control measures may represent. VanEngelsdorp's data demonstrated that amitraz is the most effective chemical control measures for *Varroa*, causing significant reduction in colony losses among the large-scale beekeepers who treated colonies with the chemical four times a year. Presentations with respect to the breeding of resistant bees focused on selection of resistant bees from colonies that exhibit robust survival rates following exposure to high *Varroa* infestation. Bees that have the ability to remove mites from their bodies via grooming have lower mite levels. Thus, selection for grooming traits in adult bees could produce bees with greater resistance to *Varroa* mites. As suggested by the mite control group, there are possible techniques to reduce mite infestation and secondary impacts through the use of new techniques that pose little or no harm to bees. These include the implementation of screen floors to delay colony infestation and the use of RNAi technology to target *Varroa* as well as pathogens carried by *Varroa*. Researchers in the *Varroa* Interaction group indicated that malnutrition and disease susceptibility are frequently associated with high levels of mite infestation. Colonies with low *Varroa* infestations are better able to keep pathogens in balance within the colony, and colonies with adequate nutrition appear better able to overcome the negative effects of *Varroa* infestation.

Abstracts

During plenary sessions on Day 1 of the summit, ten scientists from universities and the USDA-ARS presented summaries focused on examining current and recent (past 5 years) research related to the four priority areas of the conference . Below are abstracts from each of the four areas:

Varroa Biology and Effects on Colony Survival:

- a. *Varroa Population Dynamics in the US: Implications*; Dennis vanEngelsdorp; Department of Entomology, University of Maryland, USA

Varroa mites are seen as a major threat to honey bee health. Commercial beekeepers considered these parasitic mites the second most important reason that they experienced higher than acceptable losses in the winter of 2012-2013. This view is supported by survey data. Random sampling of colonies by the APHIS funded National Honey Bee Disease survey reveals that mite levels in colonies are high. A fall level of 3 mites per 100 is considered the actionable threshold (as per Genersh et al (2010)). In 2012 average mite levels exceeded actionable thresholds in 8 of 12 months, while in 2013 average monthly mite levels exceeded actionable thresholds in 4 of 12 months. The NIFA funded Bee Informed Partnership also supports the supposition that mites are a leading cause of high colony losses. Surprisingly a majority of respondent to the national management survey (59%) report not treating with a known *Varroacide* in the last 12 months; and these beekeepers lost 25% more colonies than those who did treat. These numbers are heavily biased by backyard beekeepers, as only 12% of large beekeepers (operating 50 or more colonies) reported not treating. Only large beekeepers who reported treating colonies at least 4 times reported significant reduction in colony losses, this difference was particularly pronounced among beekeepers reported using amitraz 4 times over the course of the year. Reliance on a single effective control product is of particular concern as there is evidence that other synthetic products are losing effectiveness. Clearly new mite control strategies need to be developed and communicated to the beekeeping industry to help thwart unsustainable levels of

loss.

Breeding Resistant Bees

- a. *The Journey Through Development & Implementation of Varroa-Resistant Honey Bees*; Robert Danka; USDA-ARS Honey Bee Breeding, Genetics and Physiology Laboratory, Baton Rouge LA USA

Several approaches have been used to produce *Varroa*-resistant (VR) honey bees, i.e., bees whose colonies host mite populations that remain small enough to allow beekeepers to eliminate or reduce miticide treatments. First, selection of bees from untreated 'survivor' colonies has shown some promise. While survivor bees often are not productive for beekeeping, one documented commercial success is Le Rucher D'Oc bees in France. Two other approaches have been used in North America. Selection focused on reduced *Varroa* mite population growth (MPG), combined with selection for honey production and tracheal mite resistance, resulted in Russian honey bees. Selection to enhance defined traits that reduce MPG yielded Minnesota Hygienic honey bees (with hygiene against dead brood) and bees with *Varroa* sensitive hygiene (VSH) (with hygiene against *Varroa*-infested brood, which suppresses mite reproduction). VR bees are being used successfully in North America, with some beekeeping operations having not used miticides for more than 10 years. Use of Russian honey bees is somewhat limited by queen availability, and use of VSH bees is limited by the difficulty of measuring the trait, and by lack of breeding into a recognized stock. The early adopters of VR bees tend to be dedicated, proficient, smaller-scale beekeepers. There is growing interest in using VR bees, including by larger-scale beekeepers, as these early successes are recognized. It seems likely, however, that risks associated with adopting VR technology means that extensive use of VR bees in commercial beekeeping likely will not occur until miticide treatments become unreliable.

- b. *Genetics of honey bee mite grooming behavior and attempts to breed resistant bees*; Greg J. Hunt, Department of Entomology, Purdue University, West Lafayette, IN USA

Three traits have been identified in multiple populations of bees as important for tolerance to *Varroa* mites: mite non-reproduction, removal of infested brood by workers, and grooming behavior (removal of mites from adult bees). Grooming behavior has been underutilized in breeding programs. However, progress is being made in characterizing this trait and the genetics behind it, including the identification of candidate genes through genetic mapping studies. Research has shown that the proportion of mites that have chewed appendages among those that fall to the bottom of the hive correlates with the bees' ability to groom mites off themselves. In the past six years the proportion of chewed mites in our breeding population has increased from an average of 3% to 44%. The challenges are to continue the selection, to encourage beekeepers to use the stock, and for queen breeders to perform selection for the trait. In recent years there has been an increase in interest among beekeepers for workshops on identifying resistant stock and (especially) for raising queens. We propose to initiate a community-based stock evaluation program. Beekeepers will take part in research by conducting blind comparisons between northern-bred "mite biting" stocks and commercial sources. The results from many beekeeper scientists, along with a new Midwestern queen breeders coop may help to build enthusiasm and a market for resistant queens.

- c. *Honey bee colonies (Apis mellifera) in Sweden surviving Varroa destructor infestation ("The Bond bees")*; Eva Forsgren^{1,2}, Ingemar Fries¹, Joachim deMiranda¹, Barbara Locke¹ Swedish University of Agricultural Sciences & University of Maryland

A population of European honey bees (*Apis mellifera*) surviving *Varroa destructor* mite infestation in Sweden (on the island of Gotland in the Baltic Sea) demonstrates that a balanced host–parasite relationship may evolve over time if colonies are left without mite control. This population was established as part of a natural selection experiment called the "Bond-Project" and has survived since 1999 without mite control or beekeeping management and with exposure to severe mite infestation selection pressure. Studies of this population of bees show that the overall mite population growth rate is reduced by 82% compared to control colonies, irrespective of the mite source (mites from Bond or control colonies), suggesting

that traits associated with the bees and not with the mites is responsible for reduced mite growth rate. Further studies have confirmed that surviving honey bee colonies limit the mite population growth by suppressing mite reproductive success. The surviving colonies had on average almost twice the proportion of infertile mites, more than twice the proportion of dead progeny, significantly reduced fecundity and an overall reproductive success rate of less than 50% compared with over 75% in control colonies.

Mite Control Options and Resistance Management

- a. *Mite Control Options and Resistance Management*; Keith S. Delaplane
Department of Entomology, University of Georgia, Athens, GA 30602

Control of the mite *Varroa destructor* in the USA remains centered around acutely toxic pesticides placed inside hives of living bees, an essentially risky endeavor given that the host is also an arthropod and susceptible to similar toxin modes of action. There are seven pesticides registered for *Varroa* control, and they are comprised of three synthetic active ingredients (fluvalinate, coumaphos, amitraz) and three organic (thymol, formic acid, hop beta acids). Among these six active ingredients there are concerns with reports of localized inefficacy (hop beta acids, formic acid, thymol), genetic pesticide resistance (fluvalinate, coumaphos), sublethal effects (thymol, formic acid, fluvalinate, coumaphos), or harmful synergies with other agro-chemicals (fluvalinate, coumaphos). A limited number of non-chemical IPM-friendly management options are available to beekeepers, the most promising of which are screen hive floors, genetic host resistance in bees, and trapping and removal of mites in drone brood. These IPM methods may not permanently control mites but are best used as means to delay the need to treat with an acute toxin. Beekeepers can decide when the treatable mite level has been reached with the help of published mite action thresholds, shown in North America to range from 1-2 mites / 100 bees in spring to 4-20 mites / 100 bees in August.

- b. *RNAi to control Varroa mite; Merav Gleit; Beeologics / Monsanto Corporation, St. Louis, Missouri*

It is now generally accepted that several factors contribute to the worldwide decline of honey bee health. The *Varroa* mite (*Varroa destructor*) is of particular significance. In many studies it was shown that high *Varroa* mite levels lead to colony decline. *Varroa* is the number one pest of managed honeybees (*Apis mellifera*) and a serious global threat to commercial beekeeping. Beekeepers use several types of products against *Varroa*, including chemical miticides, organic acids and essential oils. However, due to the development of resistance many of these pesticides have lost their efficacy. The search for safer and more durable alternatives is ongoing. RNA interference (RNAi) is a natural mechanism discovered in the late 1990s by Andrew Fire and Craig Mello, earning them the Nobel Prize in 2006 for this achievement. This mechanism is used by different organisms for defense from predators and regulation of gene expression and is based on a highly specific sequence recognition process. RNAi can be used to precisely target and suppress critical genes, leading to a specific pesticidal effect. Using in-vitro assays we demonstrate that *Varroa*-specific dsRNA can reduce the target gene expression and increase *Varroa* mortality, without effecting bee survival. Moreover, we show that by targeting bee viruses we can reduce virus replication and increase bee survival in lab experiments.

- c. *Biopesticides for the Control of Varroa Mites; Annett Rozek, Terramera Inc., Vancouver BC, Canada*

Terramera is an Ag-bio technology developer of sustainable pest control solutions as alternatives to traditional pesticides. The *Varroa* mite has been described as the main cause of honey bee colony decline in North America. Current solutions to treat *Varroa* mite infestations of honey bees include conventional chemicals, organic acids and botanical plant extracts. While *Varroa* mites have developed resistance against most conventional chemical treatments, organic acids and botanical extracts are effective against mites, but can cause harmful effects on bees. We have

demonstrated Proof of Concept for an application that kills *Varroa* mites on infested honey bees while only minimally affecting the bees. The active ingredient, cold-pressed neem oil, is formulated and delivered only using its vapors and avoiding direct contact with bees thus allowing control of the treatment dosage. The application will be tested in field trials this summer.

Interactions of *Varroa* with Pathogens and Nutrition

- a. *The Varroa-Virus Interaction*; Joachim R de Miranda, Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Viruses require living hosts and must ensure transmission to a new host before its current host dies. This perpetual need for transmission to survive is the main factor in regulating virus virulence. The main damage done by *Varroa*-transmitted viruses is during the pupal phase, when reproducing mites inject virus into developing pupae resulting in a >1000-fold increase in virus titre in the emerging adults. This in turn stimulates the other transmission routes, precipitating a lethal epidemic. The principal *Varroa*-transmitted viruses are the acute bee paralysis virus (ABPV) and deformed wing virus (DWV) virus-complexes. Most of the other viruses are only indirectly associated with *Varroa* infestation, as secondary infections of weakened and immunocompromised bees and colonies. The damage due to *Varroa*-transmitted viruses is exclusively due to the presence of *Varroa*: in the absence of *Varroa* these viruses return to their natural, relatively benign relationship with honeybees and are of themselves no threat to colony survival. The less virulent DWV has emerged as the preferred virus for *Varroa* instead of the more virulent ABPV-complex viruses, since the latter kill pupae too quickly, entombing the mite plus progeny. At colony level this means 2-3 years of colony survival with DWV instead of 1 year with ABPV as the main virus, and thus more opportunity for *Varroa* to disperse through robbing and swarming. Bees naturally resistant to mites are not more resistant to DWV, but are better at surviving the infection, possibly due to fewer secondary viruses and superior general health.

- b. *Varroa Mites versus Honey Bees: Altering immune responses to pathogens and interaction with nutritional stress*; Diana L. Cox-Foster; Pennsylvania State University, University Park, PA

The disease ecology of honey bee colonies is affected by multiple factors, ranging from different diseases, parasites, nutritional stress, environmental toxins, colony age structure, and genetics of the colony. In addition, neighboring colonies or other species can also have a role. Among these factors, *Varroa* introduction into the

United States has resulted in increased disease incidence, in part due to *Varroa*'s impact on bee immunity. *Varroa* parasitization can affect expression of genes associated with social immunity, cellular immunity, and anti-microbial peptides. *Varroa* also alters the hemocytes in newly emerged bees. This effect of *Varroa* on bee immunity is variable across different genetic strains, ranging from a refractory response to *Varroa* and viruses to being susceptible. Research demonstrates that *Varroa* parasitization of a pupal bee affects that bee's ability to utilize pollen as an adult. A previously parasitized bee continues to lose weight even when given a high quality diet. Altered gene expression affecting metabolic pathways underlies this effect. Functionally, a *Varroa* parasitized worker has altered physiology not compatible with being a winter bee or a nurse bee. These questions remain to be answered: How does *Varroa* impact the response to specific pathogens and to mixtures of pathogens? Is there a synergistic interaction between *Varroa* and pathogens that defeats the bee colony? Why can't worker bees recover from *Varroa* parasitization when given high quality protein diet? What is the mechanism by which *Varroa* impacts immunity and metabolism? Is there additional interplay between *Varroa*/nutrition/pathogens and environmental toxins (miticides, pesticides, and plant toxins)?

Work Group Sessions and Discussions

Day 2 of the summit focused on four concurrent facilitated work group sessions on: 1) *Varroa* biology/effects on colony survival; 2) breeding resistant bees; 3) mite control options/ resistance management; and, 4) interaction of *Varroa* with pathogens and nutrition. Attendees broke into four groups according to their preference or assignment to a particular group. During work group sessions in each group, four aspects of *Varroa* research and management were discussed: short-term goals to be achieved in 5 to 10 years; long-term goals to be achieved and established in 10 years or longer; ways to outreach to beekeeping communities; and, potential challenges for research or/and outreach. Following these discussions, the work groups presented their ideas during a second plenary session, during which they listened to ideas from others and answered questions.

Work group discussions were facilitated by USDA and EPA staff who are members of the CCD and Honey Bee Health Steering Committee. 1) *Varroa* Biology group facilitators: Dr. Jeff Pettis and Mr. Thomas Moriarty, 2) Breeding Resistant Bees group facilitators: Dr. David Epstein and Dr. Robert Danka, 3) Mite Control group facilitators: Dr. Robyn Rose and Dr. Thomas Steeger, 4) Interactions of *Varroa* with Pathogens and Nutrition group facilitators: Dr. Mary Purcell-Miramontes and Dr. Judy Chen.

Questions Posed to Break-Out Session Members

- 1) Identify the top 5-6 research priorities that need to be addressed in the near term 3-5 years and priorities to be met in the long term, more than 5 years.
- 2) What are the greatest challenges or obstacles in accomplishing these priorities, and what are possible ways to circumvent them?
- 3) Who should be the primary groups to accomplish each of these priorities (e.g. Publicly, privately, NGO funded researchers).
- 4) Identify needs for outreach (how to best communicate information on research results).

Comments from Work Groups: Each work group was provided a set of questions (Appendix 2) to help them guide a dialogue and construct future research goals and challenges. Comments from the work groups, below, do not represent the expressed opinions of agencies or personnel of the USDA, the US EPA, or other US Federal Government Agencies. These comments are not in order of priority but represent a complete list of the topics raised and discussed by each group. The Executive Summary contains a synthesis of the major themes that emerged from these discussions. Appendix 1 provides a full listing of the short-term, intermediate-term and long-term research goals, extension needs, desired outcomes and challenges to achievement of goals and needs, as determined by each work group.

Work Group 1- *Varroa* biology/effects on colony survival

Varroa has vastly changed beekeeping and requires constant attention by the beekeeper to avoid colony losses. Research into controlling the *Varroa* mite, however, should recognize that production beekeeping is not about mite-free or perfect colonies, but is about having colonies that can perform pollination services. There is a need for simple monitoring tools and treatment thresholds that are predictive of adverse colony-level effects (e.g., high viral pathogen levels, colony death, reduced honey production, etc.) Research is needed to understand how the original host, *Apis cerana*, lives relatively problem-free with *Varroa* and how we might exploit this knowledge in European honey bees, *A. mellifera*. There is much that is not understood about *Varroa* biology, etiology and epidemiology, resulting in a need for both basic and applied studies. *Varroa* is known to vector viruses, but other interactions with nutrition and pathogens are poorly understood. It is believed that basic studies of both mite and host can lead to novel control strategies.

Work Group 2- Breeding resistant bees

The group stressed that breeding priorities are primarily longer-term in nature. University and USDA scientists are primary researchers who perform breeding work

along several lines of investigation, but local stock selection by beekeepers is also occurring. Selected stocks developed by breeders are not always adopted by beekeepers, due to preferences for historically used stock and or the belief that selection for mite tolerance has come at the expense of other desirable characteristics valued by beekeepers, such as honey production or gentleness. It is true that selection for multiple traits is difficult, and that tradeoffs in desirable traits may be needed to produce a truly mite-resistant stock. Additionally, one genetic stock of bees may not serve all of the varied needs of beekeepers across broad geographic ranges in the US for pollination, honey production and overwintering capacity. Simple selection criteria should be identified, such that many small and large queen breeders can utilize the selection criteria that research has identified. Lastly, there is ongoing need for demonstration trials and extension and outreach to convince beekeepers that selected stocks have value in mite control.

Work Group 3- Mite control options/ resistance management

Predicting damage and controlling *Varroa* is an ever-changing and dynamic process, due in part to the development of resistance by the mite to pesticides used to manage them and to changing virulence of pathogens vectored by the mite (e.g. viruses). Mite management varies as to when to treat and how aggressively to treat colonies depending on many parameters including, but not limited to, climate, geographic location and other environmental stressors. In addition, each mite control material has its own use parameters and challenges. The integrated use of improved genetic bee stock, coupled with chemical and cultural control methods (e.g., mite threshold levels, control application timings, etc.) still needs to be researched under controlled field trials. Better monitoring techniques are needed to screen for mites that may be resistant to chemical control treatments. Even with improved tools to control mites, a robust outreach and extension effort is needed to convey current information to beekeepers. The workgroup emphasized the importance of mite resistance to miticides, the immediate need for new control options and the importance of creating education and

outreach programs on mites that deliver a range of best management tools and tactics that are accessible to all beekeepers and researchers.

Work Group 4- Interaction of *Varroa* with pathogens and nutrition

Varroa and its effects can vary depending on host nutrition and/or pathogen load and their interactions. Proper nutrition was seen as a basic need for honey bee colonies to resist the effects of *Varroa* infestations and/or pathogens. Land use changes across the US and the effects of weather events, such as extended droughts in principal destinations where beekeepers bring their colonies for summer honey production, has led to nutritional deficiencies for bees. Beekeepers have been feeding bees supplemental protein in recent years to offset inadequate access to diverse and nutritious forage. Workgroup participants agreed that natural forage with mixed pollen sources is best for bees. They also identified the need to develop new artificial diets that beekeepers can provide as supplemental nutrition to their colonies when natural forage is unavailable. Research is needed at the colony level to test 2 and 3-way interactions and validate any findings with longitudinal studies in the real world. For example, testing the effects of feeding artificial diets, implementing mite controls and use of hygienic or selected stock on colony growth and survival can demonstrate the relative importance of each factor. However, these studies require large numbers of colonies for adequate replication and each factor must be tested alone and in combination with each other.

Transportation of large numbers of bee colonies over long distances on large flat-bed trucks to service pollination contracts also adds stress to the colonies. Many of the landscapes to which bees are transported in order to service a number of crop pollination contracts do not provide adequate natural forage resources and proper nutrition for bee colonies (e.g., melons and cucurbits). Pollination service contracts may also place bees in close proximity to farm operations for potential exposure to pesticides. Taken as a whole, migratory colonies used for commercial pollination services are at risk of increased health challenges. Improved artificial diets and improved natural forage would provide immediate benefits to bee health. Lastly, there

is an ongoing need to convey the best knowledge BMPs to the beekeepers in a useable format.

Appendix 1. Break-Out Group Research and Extension Goals

Work Group 1- *Varroa* biology/effects on colony survival

Research & Extension Needs and Outcomes Identified:

1. Develop an understanding of *Varroa* mite reproduction in its original host and whether this can be applied to *Apis mellifera*.
2. Develop an understanding of *Varroa* mite etiology and its epidemiology in *A. mellifera*.
3. Prioritize host-parasite research at the chemical-ecology and molecular level.
4. Develop means and tools that are simple and commercially adaptable for beekeepers to monitor and predict colony health.
5. Develop tools to assist biological research of *A. mellifera* and *Varroa destructor*.

Short-term goals (3-5 years):

- Determine pathogen(s) of the *Varroa* mite, and potential side effects to bees.
- Determine predators of *Varroa* mites
- Determine *Varroa* mite resistance to acaricides and the biochemistry of *Varroa* mite resistance in species other than *A. mellifera*.
- Historical/current trends and information about *Varroa* mite etiology and epidemiology.
- Transmission dynamics of the mite.
- Population dynamics.
- Effects of current management practices of the *Varroa*.
- Practices confounding area-wide management (etiology or epidemiology of mite transmission).
- Management practices affecting mite presence (host densities or parasite virulence, prevention of swarming for commercial concerns).
- Importance of physical isolation to controlling *Varroa* mite.
- Explore effects of mite fecal deposition on *A. mellifera* reproduction.
- Determine *A. mellifera* host response to mite feeding (in the cell).
- Determine the feeding site of the *Varroa* mite.

- Effects of hive's frame foundation on mite reproduction.
- Determine the possibility of using sentinel hives to predict regional hives.
- Develop tools to monitor mite populations.

Long-term goals (over 5 years):

- Develop understanding of *Varroa* (spp.) reproduction on its host(s)
- Determine the evolutionary changes of the *Varroa* mite genome over time and *Varroa* mite host adaptations.
- Determine the population genetics of the *Varroa* mite.
- Expand modeling beyond the colony level.
- Understand local and global trends/factors that affect transmission of the *Varroa* mite.
- Understand variables that affect successful transmission between colonies (e.g. genetics, ecology, nutrition or management).
- Determine whether *Varroa* mites can be selected to suppress viruses.
- Survey original mite populations to better understand historical host(s)/vector(s) dynamics.
- Determine the key interactions that make the mite successful.
- Determine what makes *A. mellifera* so susceptible to *Varroa* mite relative to other *Apis* species.
- Determine mite population responses/changes to stressors over time.
- Improve nationwide tracking (and historical information) of *Varroa* mite and associated pathogen pressures at the colony, landscape and regional levels. The level of perspective lends itself to area-wide research.
- Compare gene expression between reproductive and non-reproductive mites in *A. mellifera* and *A. cerana*.
- Determine the molecular biology of the mite for determination of pesticide target site(s), and how site(s) differ from *A. mellifera* to not adversely affect the bee.
- Determine how variables such as cell size and the timing of colony shut-down allow bees to overwinter with *Varroa* mite.

- Consider RNAi technology as part of an IPM solution.
- Determine *Varroa* population dynamics, economic treatment thresholds and methods to interpret these data at different time points of the season to trigger action by the beekeeper.
- Determine *A. mellifera* tolerance levels for *Varroa* over time.
- Determine the spatial/temporal variables affecting mite biology, and how they affect treatment thresholds.
- Develop tools and determine methods for virus identification and treatment thresholds (keeping in mind that there may be local variations that could effect both identification and threshold levels).
- Develop models to track effects of miticides or other control mechanisms.
- Gather information from surveys or research that tracks the transmission of the *Varroa* mite between colonies.
 - Hobbyist and professionals have different management techniques that may have an effect on model research.
 - Models can be built to reflect variables that effect mite transmission (e.g., management, nutrition, ecology, or genetics).
- Artificial rearing techniques.
- *A. mellifera* cell-line development.
- Explore nano-technology as a possible delivery mechanism.
- Develop micro-array chip.
- Investigate the genome of *Tropilaelaps* mites.

Challenges:

- Funding.
- Accessible tools for beekeepers, breeders, and researchers to easily identify viral infections.

Work Group 2- Breeding resistant bees

Research & Extension Needs and Outcomes Identified:

- General desirable traits in bees selected for mite resistance: hygiene towards dead brood, increased grooming, brood effect of non-reproduction (separate from general non-reproduction that involves hygiene; VSH included here), increased ability to shed off mites, tolerance to viruses.
- General favorable characteristics in hives resistant to mite infestation: arrested reproduction of mites, reduced mite invasion of the brood, general colony survival.
- Research to consider: comb physiology that reduces mite reproduction and effects of brood post-capping duration on mite populations.
- Primary outreach audiences: bee breeders, beekeepers, researchers, extension specialists.
- Ways to perform outreach to and educate primary audiences:
 - a. Send the message up the channel through beekeeper demand (educate large-scale beekeepers so they can informally network, promote and adopt).
 - b. State/Regional and professional meetings.
 - c. Bee breeding education through online courses and social media opportunities.

Short-term goals (3-5 years):

- Develop simplified resistance measures and measurement tools for queen breeders, researchers, and beekeepers.
- Selecting and identifying key mechanisms and attributes of resistance to mites (VSH, grooming, and non-reproduction are high priority).
- Characterize genetic architecture and heritability.
- Develop infrastructure for funding tech transfer teams (on the ground diagnostics: evaluate, sample, and inspect honey bee colonies).
- Develop an inventory of desirable traits and characterizations of available genetic stock (registry for lines and what is available).

Long-term goals (over 5 years):

- Investigate marker assisted selection:
 - Identify causal genes that are driving resistance or tolerance.
 - Investigate methods for determining markers for marker assisted selection in *A. mellifera*. (Determining differences in phenotypes of behavioral versus genetic drivers.)
 - Evaluate and compare genomic differences of *A. mellifera* and *A. cerana*.
 - Develop QTL mapping, functional genomics and proteomics to identify candidate genes related to desired traits/behavioral changes
 - Investigate *A. mellifera* genetic heterogeneity to verify mechanisms from different genetic backgrounds or from isolated stock.
 - Verify how alleles work differently in different genetic backgrounds of *A. mellifera*.
- Determine mechanisms of resistance/tolerance:
 - Determine interactions between *A. mellifera* – *Varroa* and *Varroa* – virus(es) (including environmental considerations).
 - Verification of QTLs for improved understanding of *A. mellifera* immune system response to viruses.
 - Develop greater knowledge of genetic variation in bees resistant to viruses.
 - Investigate mite effectiveness at transmitting viruses.
 - Determine mechanisms unique to discrete populations versus those found in all populations (effects of regional differences, e.g., where bees are overwintering with brood breaks of more than three weeks).
 - Assessment of variation in *Varroa* Sensitive Hygiene (VSH) traits in spatially differentiated populations of bees/mites.
 - Investigate mite non-reproduction to reduce fecundity.
 - Determine what mechanisms constitute non-reproduction.
 - Develop tools to measure among breeds of breeder queens.
 - Determine the evolution of mite avirulence (ecotypes) and develop a sustainable interface between host and mite genetics to develop management practices exploiting mite avirulence, e.g.,

- Introduction of sterile *Varroa* to compete with more virulent mites present in the environment.
 - Mating disruption.
 - Breed mites that are poor virus vectors.
 - Explore possibility of producing fragile males (heterozygosity a challenge).
 - Determine the role of nutrition in *A. mellifera* resistance.
- Evaluate Genetic Stock:
 - Develop a tool(s) to facilitate productivity selection by breeders.
 - Develop methods of maintaining *A. mellifera* diversity not at odds with traits desired by commercial beekeepers (*Varroa* mite viability decreases as *A. mellifera* genetic variability increases).
 - Investigate concept of local (systems) adaptability with an eye to the needs of migratory beekeeping. Criteria include:
 - Genetically distinct from others.
 - In the stock itself and not the mites.
 - Conduct transplant experiments to identify adapted stock that does better locally (outperforms non-local).
 - Develop improved knowledge of genetic architecture: how traits are correlated and how additive is the genetic variation identified.
 - Develop improved knowledge of heterogeneity and stock evaluation (how does genetic background affect traits).

Challenges:

- Funding RFPs not favorable to needs of breeding research – difficult to maintain stocks in a university setting.
- Government cap on travel and general prohibitions on travel for federal researchers is a detriment to bee breeding research.
- Economic incentive for breeding for resistance is absent.
- Incentivizing and garnering buy-in from queen breeders to adopt bee lines with new traits (focus currently on traits desired by commercial beekeepers).
- How to give breeders tools to measure and compare with other breeders.
- How to delineate beneficial and non-beneficial traits that are often correlated.

- Variations in brood developmental period between different bee subspecies (lack of heritability of traits).
- Narrowing vs. maintaining genetic diversity.
- No purified virus(es) to conduct resistance research.
- Difficulty in establishing an isogenic line; genetic heterogeneity is an issue with any proposed program.
- Feasibility of implementing citizen science and community breeding initiatives.
- Development of methods for accurately and feasibly measuring mite loads.
- Regulations that limit advancements (e.g., international regulations).
- Researchers concern with dissemination of unwanted traits.

Work Group 3- Mite control options/ resistance management

Research & Extension Needs, Challenges, and Outcomes Identified:

1. Establish monitoring techniques and threshold development (can be accomplished by public, private companies or NGOs).
2. Perform outreach by establishing: communication within research community, i.e., publically accessible databases; communication for commercial/non-commercial beekeepers (accomplished through extension, state lead agencies, and industry).
3. Broaden, improve and enhance public/private partnerships through the introduction of new competitive grant programs.
4. Primary groups to accomplish identified priorities: stakeholders; university extension; USDA as a) facilitator (e.g., HBH/CCD Steering Committee; OPMP/EPA; ARS/NIFA for research) b) cooperators with academia in evaluating efficacy; innovation centers; resistance management (such as EPA); IPM/BMPs for tool development and extension/tech transfer

Short-term goals (3-5 years):

- Determining number of living mites.
- How best to utilize existing control options and development of new control options.

- Additional hard chemistries needed; screening existing miticides for *Varroa* control; new modes of action for resistance management.
- Beekeepers need a realistic list of BMPs for registered products.
 - Quick knock-down for short ERT chemicals where strips may provide long-term control but lack quick knock-down.
- Are current screening methods for efficacy (activity/selectivity) adequate (lab, small-scale field, full-field).
- Understanding the capabilities of individual beekeepers to implement control methods.
- Regional testing to contour treatment/control needs.
- Ensure beekeepers, etc, have adequate understanding of treatment regimen for proper use in the colony.
- Strategic approach to controlling *Varroa* since not all colonies are treated simultaneously.
- IPM (Integrated Pest Management- integrated *Varroa* management; integrated bee management).
- Effective/inexpensive monitoring; interpretation of monitoring data (regional thresholds).
- Characterizing resistance modes of action for each active ingredient.
- Develop resistance management strategies.
 - Determining appropriate delivery methods and effective integration/precision of control measures.
 - Developing lab-rearing /bio-chemical assay methods.
 - Evaluate economics associated with controlling *Varroa* including cost effectiveness of resistant queens.
 - Improve our understanding of treatment regimens for proper use in the colony.
- Monitoring
 - Determining number of living mites
 - Developing bioassay to screen for mites.

- Effective/inexpensive monitoring; interpretation of monitoring data (regional thresholds)
- Sensor technology for remote monitoring.
- IPM/BMPs
 - Improve understanding of mite population dynamics; susceptibility to acaricides; thresholds for treatments
 - Practical list of BMPs for registered products for beekeepers.

Long-term goals (over 5 years):

- New technologies
 - Selectivity for mite control without affecting bees.
 - Biological controls (e.g., RNAi)
 - Potential pheromone/chemical ecology for mating disruption.
 - Evaluate new technologies relative to population dynamics, susceptibility to acaricides and thresholds for treatments.
- Consortium with regulated community to access discovery data.
- Lab-rearing bioassay methods.
- Economics associated with controlling *Varroa*; cost effectiveness of resistant queens.
- Resistance management with greater focus on IPM (national rotation schedule) to extend life of products.
- Beekeeping represents significant challenges to IPM limiting resistance management because of heavy reliance on particular tools.
- Varroacides (screening, marketing).
- Characterizing resistance for each active ingredient.
- Effective extension program to insure adequate monitoring/treatment/IPM.
- Varroacides:
 - Defining methods for screening and selection of Varroacides.
 - Treatment regimen
 - Expedite registration process (e.g., oxalic acid registration)
 - Developing alternative pesticides (consider potential interactions)

- Developing new technologies including biochemical and biological (e.g., mating disruption; RNAi)
- Optimizing current screening methods for efficacy (activity / selectivity) adequate (lab, small-scale field, full-field)
- Extension/tech transfer
 - Developing extension and adoption technology with nation-wide access to tech transfer.
 - Creating and Identifying Innovation Institutes (public/private collaboration).
 - Developing effective clearing-house for distributing information.

Challenges:

- Proper funding and alignment of efforts, unbiased oversight/coordination/integration.
- Proper registration process for varroacides (conventional vs. biopesticides), and challenges with prohibitive regulatory costs, low economic incentive to develop products; EPA offer incentives/IR-4 involvement; as well as willingness to expedite process for conventional pesticides.
- Monitoring, and accounting for diversity in treatment needs.
- Defining uniform BMPs for beekeepers: Need for appropriate infrastructure to evaluate and implement National-level extension/tech teams; Inadequate number of tools for resistance management.

Work Group 4- Interaction of *Varroa* with pathogens and nutrition

Research & Extension Needs, Challenges, and Outcomes Identified:

1. There is a need to pull together a reliable online source, as there is a lot of conflicting information; beekeepers and public are flooded with information (and misinformation).
2. Need to establish extension programs for face-to-face communication and relay of accessible information; perhaps ability to hold, support, and host webinars accessible to many beekeeping communities.

3. Ensure all information presented by trainers is portrayed correctly and is up to date
Create an online training subscription, such as Beekeeping 101, and allocate fees toward developing high quality materials for education and extension specialists' time.
4. Possibility of keeping outreach confidential to protect beekeepers identities.

Short-term goals (3-5 years):

- Reduce nutritional stress on bees to improve general bee health (both research and action items).
- Study effect of changing transportation schedule/routes of bees on maximizing best nutritional resources; study impacts of various crop and non-crop nutritional resources for pollination optimize health of bees.
- Role of genetic diversity and id specific traits to obtain resistance/tolerance to *Varroa* and diseases (ideally internationally, but local activities).
- Identify molecular; physiological and biochemical mechanisms that *Varroa* utilizes for development and impacting life cycles in the host; this will identify targets for control methods (e.g., RNAi) and other novel approaches.
- Select *Varroa*-resistant lines ('Zena' lines, hygienic bees)
- Evaluate unique management tactics for stationary vs. migratory bees.

Long-term goals (over 10 years):

- Modeling interactions to make better predictions of interactions between mites, diseases and colony health.
- Identify treatments.
- Improve methods for nutritional diversity.
- How viruses interact with each other and how viruses evolve.
- Mechanism of resistance/tolerance to *Varroa* and diseases.

Challenges:

- Lack of funding and expertise.
- Necessity for international effort (to generate genetic diversity) could create a myriad of challenges, such as international regulation laws.

- Decide to whom, or which agencies delegate particular tasks.
- Transfer of correct information.
- Involvement in genomic data or cellular, molecular and other basic research that can be proven challenging in everyday beekeeping.
- Development of cell lines (perhaps can be achieved by private companies, such as development of hymenopteran cell line).
- Necessity for genome assembly of Varroa mite; establishment and support for interactive databases and computational expertise.

Appendix 2. Participation in Work Groups

Work Group Members

Group 1: *Varroa* Biology and Effects on Colony Survival:

Name	Affiliation	Email
Ana Cabrera	Postdoc USDA-ARS Florida	anacabreracordon@gmail.com
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Group 2: Breeding Resistant Bees:

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Group 3: Mite Control Options and Resistance Management:

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Group 4: Interactions of *Varroa* with Pathogens and Nutrition:

Name	Affiliation	Email
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Mary Purcell-Miramontes	USDA/NIFA	mpurcell@nifa.usda.gov
Colin Stewart	APHIS	Colin.Stewart@aphis.usda.gov
Tim Tucker	ABF	tuckerb@hit.net
Ethel Villalobos	University of Hawaii	emv@hawaii.edu

Appendix 3. Agenda

DAY I: Tuesday, February 18th

1:00 pm– Opening Remarks/Welcome

Jeff Pettis	Research Leader, USDA-ARS
Ann Bartuska	Deputy Under Secretary, USDA-REE
Jim Jones	Assistant Administrator, EPA
Barbara Glenn	Crop Life America
Gabriele Ludwig	California Almond Board
Brett Adee	Past President, American Honey bee Producers Association
Tim Tucker	President, American Bee Federation

2:00 pm – Presentations

I. **Varroa biology and effects on colony survival**

2:00 – 2:15 pm: Peter Neumann, University of Bern

2:15 - 2:30 pm: Dennis vanEngelsdorp, University of Maryland

II. **Breeding resistant bees**

2:30 – 2:45 pm: Bob Danka USDA-ARS Baton Rouge, LA

2:45 – 3:00 pm: Greg Hunt, Purdue University

3:00 – 3:15 pm: Eva Forsgren, Swedish University of Agricultural Sciences & Univ. of Maryland

3:15 – 3:45 pm– Break

III. **Mite control options and resistance management**

3:45 – 4:00 pm: Keith Delaplane, University of Georgia

4:00 – 4:15 pm: Merav Gleit, Beeologics

4:15 – 4:30 pm: Annett Rozek, Terramera

IV. **Interactions of *Varroa* with pathogens and nutrition**

4:30 – 4:45 pm: Joachim de Miranda, Swedish University of Agricultural Sciences

4:45 – 5:00 pm: Diana Cox-Foster, Pennsylvania State University

5:00 pm – Discussion of work groups and Closing – Jeff Pettis

6:00 pm – 7:00 pm: Meet & Greet with hors d'oeuvres at the Holiday Inn – Greenbelt
Willy K's, Terrance Room (7200 Hanover Dr. Greenbelt, MD 20770)

7:00 pm: Dinner on your own

DAY 2: Wednesday, February 19th

8:30 am – **Opening and Charge to the Work groups:** Mary Purcell-Miramontes

8:45- 11:45 am – **Facilitated work group sessions**

- 1) Varroa biology / effects on colony survival (facilitated by Pettis & Moriarty)
- 2) Breeding resistant bees (facilitated by Epstein & Danka)
- 3) Mite control options / resistance management (facilitated by Rose & Steeger)
- 4) Interaction of *Varroa* with pathogens and nutrition (facilitated by Purcell & Chen)

12:00 – 1:15 pm – **Lunch (provided)**

1:15 – 2:00 pm– **Work groups reconvene and summarize**

- Each work group group will finalize discussion and list research gaps and steps forward for presentation to the entire group

2:00 pm – **Presentation of next steps**

- Each work group group will present next steps for research to the entire group

3:00 pm– **Summary and Closing:** Mary Purcell-Miramontes USDA-NIFA

Appendix 4. List of Attendees

Last Name	First Name
Adee	Brett
Anderson	Troy
Baris	Reuben
Bartuska	Ann
Bogran	Carlos
Bradbury	Steven
Brady	Donald
Browning	Zac
Cabrera	Ana
Charest	David
Chejanovsky	Nor
Chen	Judy
Cox-Foster	Diana
Coy	Steve
Curtis	Bob
Danka	Bob
Davies Adams	Laurie
de Guzman	Lilia
de Miranda	Joachim
Delaplane	Keith
DeMarchi	Jane
Dickinson	Kimberley
Dorschner	Keith
Eagle	Venus
Eischen	Frank
Ellis	Jamie
Epstein	David
Evans	Jay
Forsgren	Eva
Garber	Chris
Garber	Meredith
Gilbert	Leslie
Giray	Tugrul
Gleit	Merav
Glenn	Barbara
Hackett	Kevin
Hansen	George
Hayes	Jerry
Hill	Elizabeth
Holy	Doug
Huang	Zach

Hunt	Greg
Hyberg	Skip
Jones	Jim
Keigwin	Richard
Krieger	Klemens
Kunickis	Sheryl
Ludwig	Gabriele
McAllister	Ray
Meikle	William G
Menchey	Keith
Moriarty	Thomas
Nasr	Medhat
Neumann	Peter
Ostiguy	Nancy
Overmyer	Jay
Pease	Anita
Pettis	Jeff
Purcell-Miramontes	Mary
Rexroad	Caird
Reynolds	Dave
Rogers	Dick
Rose	Robyn
Rosenblatt	Daniel
Rozek	Anna
Rueppell	Olav
Sagili	Ramesh
Schlegel	Paul
Schlekau	Julie
Singh	Raj
Smith	Deborah
Steeger	Tom
Stewart	Colin
Stoneman	Bill
Tarpy	Dave
Thomas	JD
Tucker	Tim
vanEngelsdorp	Dennis
Vickers	Matt
Villalobos	Ethel
Volby	Stuart
Wehling	Wayne