2008 ANNUAL REPORT ON USDA/ARS RESEARCH ON BEES AND POLLINATION

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This report is an attempt to dialogue with beekeepers on the status of ARS goals and progress in meeting those goals. ARS welcomes feedback on our goals and our progress, and welcomes suggestions that may lead us all to more quickly solve problems plaguing the bee industry.

Pages 18-19 (Appendix) of this report gives a brief summary of ARS research on colony collapse disorder. A separate report in early 2009 will address progress on colony collapse disorder among all Federal, university, and private researchers.

During 2007 and 2008, ARS honey bee research focused on a series of important problems, as delineated in our Action Plan for National Program 305: Crop Production. Listed below are the relevant Subcomponents, Problem Statements, and Research Needs from the Action Plan, which provide context for the series of Accomplishment statements presented under the appropriate Research Need.

To view the entire NP 305 **Action Plan,** please visit: http://www.ars.usda.gov/SP2UserFiles/Program/305/NP305ActionPlan-Final09-04.pdf.

Subcomponent 2A: Honey Bees

Honey bees and their colonies are threatened by a myriad of pests (including Africanized honey bees, invasive parasitic mites, and insects), pathogens, pesticides, and poor nutrition that adversely affect the health of the worker bees and their queens. These factors threaten the long-term viability of the bee industry, and the agriculture of crops dependant on bee pollination.

Problem Statement 2A.1: Improving Honey Bee Health.

Countering the effects of biological and environmental factors on the health and well-being of the honey bee hive requires a sustained, comprehensive, multi-disciplinary effort, especially given the broad scope of these factors. Parasites (especially varroa and tracheal mites), depredators that spoil hive stores (small hive beetle and wax moth), pathogens, exposure to crop pesticides and hive miticides, inclement weather, and poor nutrition all can be harmful to the hive, and in any combination, they can be devastating. In most cases, growers and beekeepers must contend with several factors in trying to keep their hives healthy and productive. In addition, because of the recent CCD outbreaks, migratory beekeeping has been unable to meet needs of almond pollination in California with domestic colonies. Bees are often moved multiple times across the country and, not uncommonly, 10 percent of colonies may be lost during each move to a new crop.

Of special concern to beekeepers is the health and robustness of the queen, which is closely linked to the health of the colony. Re-queening, an important tool of apiculture, is necessary where bee stock has become genetically weak or for making colony splits. Many beekeepers are concerned that the quality of their honey bees, especially queens and worker bees, is not as high as it should be.

Research Needs:

Development of integrated pest management (IPM) systems for parasitic mite management, including developing resistant bees, traps with lures or exclusion devices, and environmentally “soft” miticides that are non-toxic to workers and queens.

2008: New delivery system developed for essential oils to control Varroa mites. The varroa mite (*Varroa destructor*) is a worldwide threat to honey bees, and options to control them are limited. Plant essential oils show promise as acaricides against the mite, but the delivery of these compounds remains a challenge due to their low water solubility and high volatility. A technique to microencapsulate essential oils in beta cyclodextrin complexes was developed by ARS scientists at Tucson, leading to the detection of high levels of the essential oils in bee tissues without any imposed toxicity to the bees. The encapsulation technique can be used as a delivery system for many different compounds that might deter feeding and reproduction of varroa.

2008: Products developed to control pests, parasites, and diseases in honey bee colonies. ARS scientists at Weslaco tested six products, some in a dose series, against moderate to heavy nosema infections in honey bees. All were less effective than the standard dose of Fumagilin-B. Fumagilin-B, while significantly lowering spore levels, did not reduce nosema disease below the economic threshold. Only one product, Honey Bee Healthy, was comparable to the efficacy level of Fumagilin-B.

2007: Hivastan studied and temporarily approved for control of varroa in honey bee hives. EPA issued a Final Rule establishing time-limited tolerances for residues of the miticide fenpyroximate (*e.g.*, Hivastan) in or on honey. The tolerance took effect on May 9, 2007 (with objections and hearing requests due to EPA by July 9, 2007) and expires and is revoked on December 31, 2010. Since 2003, ARS scientists in Weslaco have been working under a cooperative agreement with Central Life Sciences for the development of Hivastan as a varroa mite control compound. Field trials in Texas and California showed high levels of efficacy. However, during the fall of 2006, some managed bee colonies experienced unexplained mortality during the first few hours of treatment with this product. The Weslaco unit is actively working with the registrants of Hivastan to determine what caused this unexplained mortality.

2007-2008: Beta plant acids and 2-heptanone tested for varroa mite control. ARS scientists at Tucson are testing beta plant acids for varroa mite control under a CRADA with J. I. Haas, Inc., and are formulating 2-heptanone as a miticide in collaboration with the ARS Bioproducts Chemistry and Engineering Laboratory in Albany, California. Field testing of these products began in Spring 2008 as part of the Areawide Project on Bee Health.

2007: Fungi used for control of varroa. In 2002, two fungal pathogens, *Hirsutella thompsonii* and *Metarhizium anisopliae,* were found to be highly pathogenic to the varroa mite in the laboratory and in observation hive tests, but were harmless to the bees and did not affect the fecundity of the queen bee. In collaboration with Sylvan BioProducts, Inc (fungal producing company) and Sioux Honey Bee associations, researchers have developed and tested a user-friendly delivery system to reduce mites on adult bees, improve bee hive survival, reduce numbers of residual mites, and improve colony strength in adult bee populations and brood production, and have determined optimal dosage, germination, and pathogenicity of the fungal spores to successfully control mite populations in established honey bee colonies. Overall, research shows that microbial control of varroa with *M. anisopliae* is feasible and could be a useful component of an integrated pest management program for the honey bee industry. Large scale field trials are further needed to develop a commercial fungal product for the honey bee industry.

2007: Microbial control of varroa using fungi naturally occurring in beehives. In 2005 scientists at the ARS laboratory in Montpellier, France, isolated and identified naturally-occurring strains of *Beauveria bassiana*, a known insect pathogen, from varroa mites. The fungus was formulated into a binary compound containing plant wax powder, and the resulting biopesticide was shown to increase varroa mortality with no observed negative impact on colony health. Since then, experiments have been conducted using different kinds of plant wax powder and with multiple treatments to assess mortality and fungal dosage. A patent for the biopesticide was filed in November 2007, and further work is being conducted to optimize dosage and number of applications. This research has attracted the attention of the European bee industry.

Determination of the role of varroa mites in suppressing bee immunity and in vectoring bee viruses, especially the appearance of virus disease long after varroa is eliminated by miticides.

Development of additional methods (*e.g*., lure traps) to protect hives and hive stores from small hive beetles in "honey houses," which will also help in monitoring the beetles in the field.

2008: Comparatively fewer small hive beetle found among Russian honey bee colonies than Italian colonies. Small hive beetles (*Aethina tumida*) (SHB) began appearing in U.S. hives during the past 15 to 20 years and now infest bee colonies throughout the East. The comparative resistance of Russian colonies to the SHB was unknown. Studies done by ARS scientists in Baton Rouge showed that Russian colonies carried fewer SHB adults in their colonies, especially if entrance reducers were used in hives. This study provides a specific management tool for beekeepers to help control SHB.

2007: A new way to lessen damage from small hive beetles developed for honey bee colonies. ARS scientists in Gainesville have developed an apparatus and attractant to help beekeepers protect their honey bees. Small hive beetles deposit a yeast that is highly alluring to fellow beetles. When the yeast grows on pollen in the hive, it attracts more beetles and sets off a cascading effect; disturbed bees leave the hive. To exploit the small hive beetle's biology, scientists installed traps baited with the yeast below test hives belonging to cooperating beekeepers, which allowed the beetles to enter the traps, but not to exit. These traps may solve the problem for small-scale beekeepers, which make up 60 percent of the industry. A patent for the trap was filed in March 2005. For large-scale beekeepers who maintain up to several thousand hives, researchers hope to develop a new trap requiring less management. If perfected, this trap could be a boon to the bee industry in Florida, which is a common overwintering destination for commercial bee colonies. Researchers hope to apply the same principle to reduce populations of varroa mites, another significant pest in honey bee hives.

Determination of the level of honey bee stock resistance to parasites and pathogens, identification of bee genes, traits, and markers associated with resistance, and breeding of bees with improved resistance.

2008: Genetic diversity of Italian bees in the United States found to be similar to the diversity of Italian bees in Italy. There was a concern that importation and breeding bottlenecks had endangered U.S. bee stocks through inbreeding, but a full survey conducted by ARS scientists at Baton Rouge showed that the diversity of Italian honey bees in the United States is quite high. This provides guidance that there is no need to attempt to enrich the genetic diversity of Italian stocks by importations from Italy.

2008: Full complement of Russian bee breeder lines released to the beekeeping industry. The need exists to provide a final transfer of all Russian bee breeder lines to industry. Working with the CRADA holder and a newly formed Russian Bee Breeders Association, ARS provided members systematically with breeder queens for queen lines and for drone sources so that each member could produce two of the eighteen lines. In the future, members will share stock to develop new drone source colonies and maintain their ability to develop all of the lines. This will permit the continued breeding and selection of Russian bees and provide mite-resistant Russian honey bee stock to the entire beekeeping industry for the foreseeable future.

2008: Successfully requeening Italian colonies with Russian honey bees. Worries have been expressed that it is difficult to introduce Russian queens to Italian colonies. ARS scientists at Baton Rouge found that Russian and Italian colonies accepted both mated Russian queens and Russian queen cells equally well, and that higher but not economically damaging levels of varroa infestation reduce the rate of requeening success. This finding provides beekeepers with information to produce colonies with desired queen stock and increase their rate of success in managing commercial apiaries. These tools are being employed by many beekeepers throughout the country.

2008: The suppressed mite reproduction (SMR) trait found to be caused by removal of mite-infested brood cells by adult bees. Understanding the behavior of bees that resist varroa mites will help breeders to test and select for resistance. Field testing by ARS scientists in Baton Rouge showed that bees with SMR apparently removed mites from capped cells; thus, the trait was renamed varroa-sensitive hygiene (VSH). Of the mites that remained, VSH colonies had a much higher rate of nonreproductive mites (80%) than non-VSH colonies had (29%), suggesting that VSH bees removed reproductive mites more often than they removed nonreproductive mites. By knowing the mechanism behind the VSH trait, scientists and bee breeders will be better able to select for this trait and also separate this trait from other mite-resistance traits.

2008: Extensive recapping of worker brood cells by VSH bees identified as screening mechanism. Improving understanding of the biology of VSH may help breeders in testing and selecting for varroa resistance. Researchers found that differences between VSH and susceptible bees in the frequency of recapping of brood cells tend to be greater than differences in other measures related to mite resistance. In addition, recapping is easier to identify than other measures of mite resistance (*e.g.*, the reproductive status of varroa). Recapping frequency thus has been identified as a simple screening tool that correlates to the expression of VSH by a colony.

2008: Italian X varroa sensitive hygiene (VSH) germplasm developed. ARS scientists in Baton Rouge sought to combine bee lines having high VSH with lines of commercial Italian stock, yielding some lines with good beekeeping characteristics and that also had low varroa population growth. The best performing colonies are being propagated to develop a semi-closed population of VSH germplasm for future release to industry. When completed, this germplasm production should improve adoption of mite resistant bees by commercial beekeepers involved in crop pollination.

2008: Russian honey bee resistance to tracheal mites found to result from autogrooming. The mechanism of honey bee resistance to tracheal mites was uncertain, but work by ARS scientists at Baton Rouge has confirmed that the primary mechanism of resistance is autogrooming by very young adult bees. This advance makes it possible to directly phenotype breeding stock and potentially develop marker-assisted breeding.

2008: Varroa resistance and general beekeeping utility of VSH honey bees demonstrated. Field studies are needed to document the utility of mite-resistant bee stocks. VSH and Russian bees developed by ARS scientists at Baton Rouge were tested in cooperation with beekeepers in Alabama. Through three seasons of measurement, resistant stocks required less treatment against parasitic mites than the Italian-based control stock did, and the overall average honey yield from Russian (59 lb) and VSH (52 lb) colonies was comparable to that of control colonies (46 lb). Beekeepers did not report any significant behavioral problems with resistant stocks.

2007: Vertical and horizontal transmission of bee viruses demonstrated. ARS scientists in Beltsville demonstrated both vertical (adult to brood to adult) and horizontal (bee to bee) transmission routes for virus movement in colonies, and bee viruses were also demonstrated to be present in pollen. A better understanding of virus transmission routes will help in designing ways to mitigate the effects of bee viruses on colony health.

2007: Studies initiated to complement the VSH trait by developing another varroa-resistance trait, brood-induced suppression of mite reproduction. Although ARS scientists in Baton Rouge have demonstrated that almost total resistance to varroa mites can be achieved with only the VSH trait, it is not prudent to rely on one mechanism for mite resistance. Recent observations in Baton Rouge build on prior reports that brood in some colonies may directly suppress the number of offspring produced by foundress varroa mites. If heritable effects by brood on the reproduction of varroa can be demonstrated, selective breeding could enhance this trait. Research is underway to team brood effects with VSH to broaden varroa resistance.

Improvement in molecular tools for assessing the effects of diet, management, and other stressors on the abilities of bees to resist disease.

2007: Honey bee genome sequenced and published. The sequencing of the honey bee genome was published in *Nature* this past year and reflects an enormous effort by the scientific community. ARS scientists in Beltsville, working with those in Weslaco, were instrumental in the conception and completion of this accomplishment. The full impact of the genome has yet to be realized, but recently the genome was used to analyze bees from CCD-associated colonies for exposure to pesticides and diseases in an attempt to uncover the causes of the disorder. The honey bee genome will open new opportunities to explore bee health, behavior, and physiology, and in turn improve beekeeping and pollination.

Establishment of bee cell lines.

2007: Effort initiated to establish bee cell lines. Scientists working with viruses at the Beltsville laboratory are working to establish cell lines from bees. This will enable cultivation of bee viruses (*e.g.*, IAPV), potentially important for understanding factors impacting CCD and therefore making progress in controlling the disorder. Funding issues have recently reduced prospects for recruiting a full-time scientist to lead this work.

Improvement in the sensitivity, ease of use, and cost of disease diagnosis.

Determination of improved management to produce larger colonies for early pollination.

2007: Relationship between honey bee colony size and composition shown to be a factor in almond pollen collection. In cooperation with the California State Beekeepers Association, ARS scientists in Weslaco investigated honey bee colony size and composition as factors in almond pollen collection. They found that Australian package colonies performed better than they did during 2006, but not as well as 8-frame U.S. colonies, and 4-frame colonies collected even more pollen than either the 8-frame or Australian colonies.

2007: Russian honey bee colonies found to be larger in winter after developing in the fall in 8-frame hives and being fed Tucson bee diet. ARS scientists in Baton Rouge compared the growth of Russian honey bee colonies that had been established in August in 8- or 10-frame hives, half of each group having been fed the Tucson bee diet in early November. In December, colonies originally in 8-frame hives had 1.6 more frames of bees than did colonies originally in 10-frame hives (8.6 vs. 7 frames of bees). Colonies that were fed had ½ of a frame more brood than colonies that were not fed (1.5 vs. 1.0 frame).

2007: Worker flight activity of Russian and Italian honey bees shown to be equal during both blueberry and almond pollination. Comparative experiments by scientists in Baton Rouge showed that worker flight activity of both Italian and Russian colonies was equivalent during almond and blueberry pollination. Forage rate was similarly influenced by colony size, temperature, and time of day.

2007: Sunflower seed set increased by honey bee pollination. In a study on the pollination of self-compatible sunflowers, the ARS Tucson Laboratory found that honey bees increase seed set when they forage on sunflowers and that they can mitigate the effects of high temperatures on seed yields.

Determination of the effects of miticides on bees and the impact of pesticides on foragers, both through lethal (acute) and sub-lethal (chronic) exposure.

Development of miticide-resistance management programs, and procedures for reducing bee exposure to pesticides.

Determine the effects of nosema on colony growth.

Determination of best management practices for pollination migratory beekeeping, including the acceptable number of transports and proper treatment of bees during and after transport.

Identification of nutritional factors that stimulate colonies to rear brood, and determination of the effects of nutrition from pollen, artificial diets, and nectar substitutes (*i.e.*, protein and sugar) on bee health, pollination rates, and honey production.

2007: Queen-specific volatile compounds identified. The Tucson laboratory identified queen-specific volatile compounds that play a role in queen acceptance, particularly when European queens are introduced into Africanized colonies.

Identification of signals (particularly chemical cues) provided by larvae to nurse bees that stimulate feeding to become queens.

Development of feeding regimes for colonies so that nutritional needs are met for rearing queens and drones.

Characterization of the microbial associates of healthy honey bees.

2007: Typical bacterial complement defined in bees. ARS scientists in Beltsville, along with university colleagues, demonstrated a surprisingly consistent set of bacterial associates in honey bees, showing that 10 bacterial species are nearly universal across continents and bee subspecies. The results have bearing on determining the impacts of non-pathogenic bacteria for bee health and nutrition.

Problem Statement 2A.2: Pollination of Crops.
Honey bees are essential for the seed and fruit set of crops comprising one-third of U.S. agriculture. Colonies often pollinate several different crops during the same year, beginning in early spring and ending in late summer. Pollinating some crops (e.g., melons, cucumbers, and blueberries) is extremely stressful to honey bees because there is relatively little pollen and the crops lack nutritional value for the bees. Colonies are weakened and often cannot be introduced for pollination of other crops. The colonies need to be intensely managed or combined with others to achieve large enough populations to survive the winter. Weakened colonies also are more susceptible to disease and impacted more by parasitic mites. Nutritional stress on colonies might be alleviated with supplemental feeding of protein while colonies are in the field for pollination.

Accomplishments include:

2008: Honey bees found to collect large amounts of upland cotton pollen in Louisiana. In dry areas honey bees do not collect upland pollen, but such pollen collection had not been studied in more humid areas. ARS scientists at Baton Rouge discovered that honey bees do in fact collect upland pollen, creating the possibility of fostering the selection of hybrid cotton using honey bees as pollinators.

2008: Worker flight activity of Russian and Italian honey bees shown to be equal during both blueberry and almond pollination. Scientists believed that there were potential differences between the ability of Russian and Italian stocks to pollinate during cooler weather. However, ARS scientists at Baton Rouge found that both Russian and Italian stocks were equally useful for commercial pollination in the same weather conditions. This finding is instrumental for promoting the adaptation of Russian honey bees for commercial pollination.

Research Needs:

Development of methods for providing protein supplements to colonies when pollinating crops that are nutritionally inadequate.

2008: Impact of synthetic brood pheromone (SBP) on almond pollen collection studied. ARS scientists at Tucson conducted field trials near Bakersfield, California, to investigate bee colony size and composition as factors in almond pollen collection. They found that Superboost (synthetic brood pheromone) increased pollen collection only on small 4-frame colonies that were used for trapping pollen but not in other colony types. This failure of other colony types to collect more pollen was caused by the bees actively managing the pollen storage area near the brood nest and the ease of replacing consumed pollen. Scientists concluded that in the area where the bees actively manage for pollen storage, adding brood pheromone is unlikely to increase almond pollen foraging. This suggests that the use of SBP may be appropriate for treating colonies on crops with relatively low pollen levels (*e.g.*, blueberries and cucurbits), as bees are unlikely to harvest enough pollen from them to provide a pollen storage area.

2007: Tucson bee diet developed for commercial production. Honey bee colony population growth and survival depend on nectar and pollen from flowering plants that might be unavailable due to weather or the movement of colonies for pollination. To provide nutrition when flowering plants are unavailable, ARS scientists in Tucson formulated a supplemental protein diet known as the Tucson Bee Diet, which is comparable to naturally collected pollen in attractiveness to bees, consumption rates, and in stimulating colony growth. The Diet has gone into commercial production, and promises to be an important component in addressing the impact of poor nutrition on colony health and in preventing CCD brought on by insufficient amounts of pollen in the hive.

Determine the effects of supplemental feeding on foraging and pollination rates.

2007: Artificial diet shown to help colonies meet 6-frame strength criterion. In cooperation with Vita (Europe) Limited, ARS scientists at Weslaco investigated the effects of supplemental feeding on overwintering honey bee colonies infected with *Nosema ceranae*. They found that in nosema-infected colonies that were fed an artificial diet supplemented with pollen, 85% of those colonies met the 6-frame strength criterion for almond pollination. However, for colonies that were not fed, only 36% met the criterion.

Problem Statement 2A.3: Developing and Using New Research Tools: Genomics, Genetics, Physiology, Germplasm Preservation, and Cell Culture.

The U.S. beekeeping industry faces numerous challenges that may be addressed by traditional genetic approaches and with emerging, molecular-based, technologies, based on the recently sequenced honey bee genome. Using genomics, scientists can link genes with desired traits, using adjoining 'markers' that indicate important genes or even variants within genes themselves that have a direct impact on health or behavior. This technology allows a strategy called marker-assisted breeding that should speed the development of new lines useful to the beekeeper. Once the markers are identified, preserving germplasm is central to efforts to improve honey bee stock. An ability to preserve desired bee lineages – as sperm, eggs, or embryos – could provide beekeepers with more time to assess the traits of their bees before making breeding decisions. Long-term preservation offers an opportunity to keep desired traits indefinitely and to transfer these traits among bee breeders.

Research Needs:

Development of molecular tools for identifying bee strains, subspecies, and stocks (lines) of interest.

Knowledge of the honey bee genome (genes, alleles, and markers) that enable researchers to make progress toward selecting for desirable traits, and development of protocols to screen germplasm for desirable or undesirable traits.

Development of techniques for non-invasive assessment of colony stress (*i.e.*, via development of a "bee health chip" microarray).

Development of molecular tools, including bee cell culture, for studying pathogen invasion and bee defense against common bee diseases, including American foulbrood and chalkbrood.

2007: Genome analysis completed of the honey bee bacterial pathogen *Paenibacillus larvae*. ARS scientists have identified sequences encoding for bacterial virulence factors (including toxins) from the genome of the honey bee pathogen *Paenibacillus larvae*, causative agent of American foulbrood. Considering the recent emergence of antibiotic-resistant *P. larvae* bacterial strains, the identification and understanding of toxins produced by this pathogen is increasingly important and will potentially lead to development of antimicrobial drugs that disrupt the process of host invasion and prevent establishment of the disease.

Additionally, ARS scientists at Weslaco discovered a molecular mechanism underlying resistance of *P. larvae* to oxytetracycline (OTC) antibiotic. Scientists isolated and completed sequence analysis of a small circular plasmid (pMA67) from *P. larvae* strains resistant to OTC, revealing that the plasmid carries a tetracycline resistance gene (TetL). The discovery of the TetL gene found in resistant *P. larvae* versions may help with the development of effective new antibiotics.

2007: Understanding of chalkbrood increased. Chalkbrood disease of honey bees is caused by a fungus, *Ascosphaera apis*, that infects larvae. The main source of this fungal infection is sexually produced spores that can contaminate all parts of the beehive. ARS scientists at Weslaco used a genome-wide approach to identify and analyze elements of a pheromone-signaling pathway in this honey bee fungal pathogen. Molecular dissection of this signal-transduction pathway will increase our understanding of sexual reproduction in this bee pathogen and provide a platform for therapeutic intervention in different disease states, including infection.

Better understanding of genes and proteins controlling queen-worker developmental differentiation.

Reliable long-term storage methods for sperm and embryos.

Determination of factors involved in poor mating and survival of queens.

2007: Queen supersedure rates shown to increase as varroa mite infestations increase. In recent years, beekeepers have noticed increases in rates of queen loss in colonies when attempting to introduce new queens to colonies. The colonies usually rear their own replacement queens, but costly queens of a desired stock are lost and the colonies develop more slowly and are less productive. A study by ARS scientists at Baton Rouge showed that higher rates of varroa infestation resulted in higher rates of queen loss. Colonies that lost queens and replaced them with queens they produced themselves were smaller and, at the end of the queen replacement process, were more highly infested with varroa. This finding shows that beekeepers can reduce queen losses by controlling varroa mites prior to attempting to install new queens and supports the recommendation that they target colonies suffering queen loss with early treatments to control varroa.

Subcomponent 2B: Non-*Apis* Bees.

Non-*Apis* bees have been utilized as pollinators of crops and continue to play a critical role in maintaining native flowering plants that form the basis of our wild land ecosystems. To maintain the productivity and the health of these bees and expand non-*Apis* bee species use in crop production, there needs to be a greater understanding of the physiology and behaviors of select species of interest to growers and the bee industry, including the alfalfa leafcutting bee (*Megachile rotundata*), the alkali bee (*Nomia melanderi*), the blue orchard bee (*Osmia lignaria*), and bumble bees (*Bombus* spp.).

Problem Statement 2B.1: Management for Crop Pollination.

If non-*Apis* bee species (whose lifestyles range from solitary to social) are to play a greater role in crop production, more information is needed on their stewardship, including their habitat requirements, husbandry, handling and over-wintering storage, disease and health issues (chalkbrood disease and unknown causes of immature mortality), and the role that chemical cues play in finding nests or appropriate forage. While some of these areas of concern have received much study in the honey bee, less has been done on the various non-*Apis* bee species. Better knowledge in these areas should lead to improved management strategies.

Accomplishments include:

2008: A new bee identified to pollinate cane berry fields. Cane berry producers have sought ways to lessen their dependence on honey bees for pollination because they are often relatively small growers without access to migratory beekeepers. A manageable native bee in the genus *Osmia* has been identified by ARS scientists in Logan as a good berry pollinator, and ARS has now developed methods to successfully use this bee for commercial cane berry pollination, including methods to increase its population levels, completing 5 years of successful field testing and modification. This bee is now being used by some Oregon farms as well as in California. Populations are being increased 2 to 3-fold per year in commercial berry fields using nesting materials that are readily available to growers.

2008: Effect of fire on native bees. Fires represent a major ecological force in the West, although their effects on pollinators is poorly known. ARS scientists at Logan conducted a 2-year study in two widely separated localities, Yosemite National Park and Zion National Park, and compared pollinators in adjacent plots of burned and unburned forest and shrub land. In both parks, the abundance and diversity of bee pollinators was significantly greater in the burned areas. Burns therefore appear to represent opportunities for increasing pollinator populations, and as such, may be important ecological events for maintaining wild bee diversity.

Research Needs:

Determination of the effect of cell handling (*e.g.,* rough physical treatment during wintering, incubation, and release) on establishment of alfalfa leafcutting bee and blue orchard bee nests.

Determination of stocking densities for the alfalfa leafcutting bee and alkali bee, both in terms of providing optimal pollination as well as maximum bee build-up.

2007: Native pollinator shown effective for alfalfa seed pollination. A large-scale, multi-year field experiment on the population dynamics of the alkali bee, a native, ground nesting bee used for alfalfa seed production, was completed in cooperation with Washington State growers. ARS scientists at Logan determined that 3 million nesting females from one nesting bed can readily pollinate 160 acres of alfalfa, yielding 1000 lbs of clean seed per acre. Moreover, seven years of sustained population growth across the watershed resulted in 16 million nesting females. This project has demonstrated that the alkali bee can be a sufficient pollinator, alone, for alfalfa seed production. It has now become the primary pollinator for the region of the study, pollinating several million pounds of alfalfa seed annually at a fraction of the management cost for alfalfa leaf-cutting bees.

Evaluation of nesting establishment and orientation cues (including chemical attractants) that mediate dispersal and nesting behavior of alfalfa leafcutting bees and blue orchard bees.

2008: Nest recognition for a pollinating bee species. The use of olfactory cues for nest recognition by the solitary alfalfa leafcutting bee, *Megachile rotundata* (Hymenoptera: Megachilidae), was studied in a greenhouse environment by ARS scientists at Logan. Findings provide evidence that the bee Dufour's gland plays a role in providing olfactory cue compounds for nest recognition; this work may lead to better bee management strategies.

2007: Blue orchard bee incubation box shown to improve bee emergence in orchards. Blue orchard bees are excellent pollinators for tree crops such as almonds, cherries, apples, and pears, and a relatively small number of bees are needed for these crops. However, fruit trees bloom early in the spring when temperatures can sometimes be cool enough to inhibit bee emergence from the winter cocoon stage and reduce blue orchard bee activity. ARS scientists in Logan designed and field-tested a new outdoor incubation box, compared emergence rates of bees incubated in the boxes with those incubated under standard conditions, and determined that the boxes facilitated faster bee emergence without increasing mortality. The new incubation box will be an effective tool for shortening emergence periods, thereby improving management of these bees when weather is variable and unpredictable. A patent application for the box has been filed, and a customer has expressed interest in licensing the patent once it is available.

2007: Old nest components attract female alfalfa leafcutting bees and blue orchard bees. These two solitary bees are used by farmers for pollination of alfalfa seed and tree fruit crops, respectively, and it has long been recognized by the growers that these bees prefer to nest in or near previously used nesting materials. ARS scientists in Logan found that volatile compounds emanating from leaf pieces used by the alfalfa leafcutting bees to line their nests and feces attached to the cast-off cocoons were attractive to nesting leafcutting bees, but other nesting materials were not. For the blue orchard bee, volatile compounds from cast-off cocoons and the paper straws we use to line the nest cavities were the attractive materials. Scientists also found that, for these two bee species, actively nesting bees excrete chemicals that they use to mark and identify their own nests. Understanding that solitary bees are attracted to particular chemical components for nesting is the first step in developing attractants that could be manufactured and applied to nests to encourage the bees to nest in desirable places, and to reduce the absconding of economically-important bees.

Determination of the role of chemical cues in parasite attraction to nests to assure that attractants used artificially do not increase parasites.

Investigation as to the cause and management of a condition called "pollen balls" in alfalfa leafcutting bees.

2008: Weather conditions correlated with bee losses due to pollen ball syndrome. "Pollen balls" (PB) is a condition in the alfalfa leafcutting bee where a fully-intact pollen and nectar provision (as provided by the mother bee) is left in the nest cell at the end of the season. This condition, accounting for 60% of reproduction losses in commercial populations, occurs when the egg or very small larva dies, or if an egg was never laid on the provision. ARS scientists at Logan collected alfalfa leafcutting bee nest cells over a three year period from several seed producing states and from Canada. Scientists found that in Canada, PB cells were more common in cool, wet weather, and most contained mold. In the United States, where PB was more common, most PB cells were not moldy, and were more common in fields with more hot days. Scientists also found that most cells with no larvae or dead eggs were present under cool conditions, while cells with larvae dying at a very young age were more common in very warm weather. Therefore, PB has been correlated with extreme weather conditions, indicating that growers should moderate the moisture and temperature fluctuations in their hives as much as possible.

Development of a better understanding of the diversity of the *Ascosphaera* fungi (which causes chalkbrood disease) using molecular systematics.

2007: New genes being sequenced for several species of chalkbrood. ARS scientists have been sequencing the genes of 15 *Ascosphaera* (chalkbrood) species (including 25 strains) and three other fungal species. In the past year, ARS scientists at Logan have resolved several sequence ambiguities, completely sequenced the EF1 α genes from 10 species, and extended the sequence analysis for the other strains. Scientists also obtained the ITS sequence for each of the strains to allow comparisons to prior work. Results will be used to evaluate the phylogeny (evolutionary history) of the genus.

Determination of the modes of disease transmission of alfalfa leafcutting bee to *Ascosphaera*, including multiple infections, disease reservoirs, and determination of the genetics of chalkbrood resistance.

2007: Study on *Ascosphaera* (chalkbrood) infections in alfalfa leafcutting bees concluded this year. ARS researchers collected seeds from growers throughout the western United States and used DNA markers (using species-specific primers) to evaluate the chalkbrood species causing infections and the commonality of multiple infections. The percentage of diseased bees that were infected with chalkbrood ranged from 34-86%, depending on the seed grower, with a mean of 61%. The majority of chalkbrood infections were caused by one pathogen, *Ascosphaera aggregata* (95%). Other species found were *A. proliperda, A. atra,* and *A. pollenicola*, but these were mainly found as co-infections with *A. aggregata.*

Development of molecular tools for studying *Ascosphaera* infection and immune response of the alfalfa leafcutting bee during development of chalkbrood disease.

2008: Little similarity shown between immunity in leafcutting bees versus honey bees. The honey bee genome has been sequenced and used to identify important immune response pathways. ARS scientists at Logan have now identified and sequenced genes for the alfalfa leafcutting bee related to chalkbrood infections. In comparing these genes to honey bees, only a few genes have been found in common. These results are added evidence that honey bees have a relatively unique immune system among the insects. The improved scientifically based understanding of immunity in bees will lead to improvements in disease control in these beneficial insects and assist scientists in developing alfalfa leafcutting bees that are more disease tolerant.

2007: Microarray of immune response genes under development. DNA markers using species-specific PCR primers have already been developed, and this year ARS scientists at Logan began developing a microarray of immune response genes in the alfalfa leafcutting bee when challenged with *A. aggregate* (chalkbrood); the EST libraries are underway. The microarray will ultimately be used to compare different populations of the bee for immune response and to test the effects of environmental factors such as stressful temperatures and sub-lethal pesticide exposure.

Identification of methods for controlling chalkbrood, including fungicides and disinfection strategies for nesting material and shelters. Methods should be robust, such that they can be applied to other solitary bees.

2008: Chalkbrood disease in alfalfa leafcutting bees demonstrated to be controllable with fungicides. Alfalfa leafcutting bees are typically stored over winter as cocoons, and ARS scientists at Logan determined that partial control of chalkbrood disease can be achieved by treating the cocoons with fungicide when the bees are warmed in the spring before being released into the field. To achieve disease control, nesting boards must also be disinfected and used in combination with fungicide treatments. Seed producers have not been able to control this disease in the field, and this method provides the most substantial control yet offered.

2007: Ozone tested as a possible fumigant for treating nesting materials and bee hives when they are in storage. ARS scientists at Logan found that high concentrations of ozone (250-500 ppm) were capable of killing all stages of wax moths and for degrading pesticides. Methods are still being developed to evaluate the effectiveness on chalkbrood spores, which is more difficult.

Development of management systems for mass production of blue orchard bee for California almond pollination, and their relatives for other crop applications.

2007: Patent filed for an in-field incubation system that can be used to trigger early emergence of blue orchard bees, making them available for almond pollination. Four species of flowering plants were tested by ARS scientists at Logan for their ability to serve as alternative pollen and nectar sources for the blue orchard bees after almond bloom has finished. Research was initiated to determine the heritability of local adaptation by these bees. Current supplies of blue orchard bees come from northern states. The bees will fly and pollinate almonds orchard adequately, but the larvae produced are not adapted to the long California summers and short winters. Field testing this strategy began this year.

Identification of bumble bee species native to the western United States amenable to artificial propagation, and that might be used to augment open field pollination.

2007: Work initiated to identify bumble bee species amenable to artificial propagation. In the first year of the study, queens from ten different bumble bee species were collected by ARS scientists at Logan, who identified two that were readily propagated.

Development of production systems for managing bumble bees in portable nesting boxes for crops produced in enclosed systems.

2008: Western bumble bee species developed as greenhouse pollinators. Bumble bees are used for pollination of greenhouse crops, such as tomatoes and peppers, reducing pollination costs considerably over hand pollination. Unlike honey bees, bumble bees are well adapted to greenhouse environments, but there are no species commercially available in the Western region. ARS scientists in Logan have identified three Western bumble bee species with good production potential, and queen production has been improved from less than 10% in 2007 to more than 25% in 2008. Thus, these species show great potential to meet the need for a western bee that can be mass propagated.

Evaluation of diseases and parasites in bumble bees.

2007: Collection of bumble bee queens. When queens failed to form a colony in confinement, ARS scientists at Logan stored them for later evaluation of disease status.

Problem Statement 2B.2: Bee Biodiversity and Contribution to Land Conservation. As with crop production, there is growing concern about the sustainability of pollination services in natural landscapes. Land managers in the western United States are in critical need of affordable native plant seed for revegetation of rangeland and wild lands, due to the destruction of native plant communities from invasive weeds, natural disasters, and human activities. Farm production methods for wildflowers could provide an affordable seed source, and in the quantities necessary for revegetation efforts, but information is lacking on what bee species (if any) are needed and how to acquire and manage these bees on a commercial scale. In addition, the 2007 NAS report documented specific examples of bee deficits, including the possible extinction of the Franklin bumble bee and the disappearance of the bumble bee *Bombus occidentalis* in parts of its native range. Inadequate systematics capacity was also mentioned. While the study team suspected declines of other wild bee species, they indicated that any such analysis was greatly limited by lack of data. The need to facilitate conservation of bee biodiversity and develop pollinators for land restoration is rooted in expanding our knowledge of bee systematics in agriculture and natural systems.

Research Needs:

Broad, base-line surveys of bee diversity in public rangelands and national parks, and the characterization of bee pollinator guilds in wild lands where bees will be critical to sustaining restoration efforts.

2007: Bee diversity in public rangelands and national parks assessed. ARS scientists from the gene flow laboratory in Madison, Wisconsin, have been collecting data on bee abundance on the Rocky Mountain columbine in different national parks and public lands in the southwest over the last seven years. The resulting assessment of bee diversity will help future conservation efforts.

Determination of the status of native bumble bee populations and evaluation of factors that threaten the health of wild populations and the identification and evaluation of other sensitive bee species in wild lands.

2007: Bumble bee genetic markers developed to evaluate population health. Increasingly, questions arise regarding whether our native pollinators are declining, particularly our native bumble bee populations; however, little work has been done to develop the tools necessary to study the abundance and diversity of native bees. ARS scientists at Logan developed a protocol for using DNA methods (based on microsatellite markers) to determine the population genetics for 32 species of native bumble bees. These protocols can be used to reveal the genetic diversity and number of bumble bee colonies in an area based on a sample of collected bees. Additionally, these genetic markers have proven useful as taxonomic characters to identify difficult species (male bumble bees are particularly difficult to properly identify), improving our ability to correctly identify species. These markers allow researchers to evaluate whether native bumble bee populations are declining with only a few years data, rather than requiring several years of extensive sampling.

Revision of key taxa in the bee family Megachilidae.

2007: *Anthidiini* taxonomy revisions progressing. Further progress was made on a major revision of the *Anthidiini* be family.

Development of new techniques to assist in easier identifications of bees for non-experts (*e.g.*, using imaging systems and interactive keys**)**.

Maintenance and expansion of the U.S. National Pollinating Insects Collection, including the associated Web-based database.

2007-2008: Bee identification enhanced. Bees are currently undergoing rapid declines, and some species are going extinct. A rapid assessment of our native pollinators is much needed, but the inability to rapidly and accurately evaluate bee status presents a major roadblock. Currently, in the United States and the world, only a few experts in bee taxonomy are competent to provide such identifications. ARS scientists at Logan have worked in collaboration the Patuxent Wildlife Refuge to develop Web-based identification guides to the bees of the Eastern United States and a guide to the genera of Megachilidae, a prominent bee family of North America. These guides allow non-specialists to accurately identify bees and expand our National capacity to conduct pollination research and preserve threatened bees.

Identification of native forbs with potential for commercial seed farming that can be used to restore wild lands, discovery of their best pollinators, and development of bee management strategies for pollinating these forbs.

2007: Restoration of the Nation's rangelands begun. Federal, State, and private land managers in the western United States are critically in need of affordable native plant seed for revegetation of rangeland and other wild lands due to the destruction of native plant communities from invasive weeds, natural disasters, and destructive human activities. Farm production methods for wildflower seed could generate restoration seed at affordable prices; however, in order to produce seed on farms, pollinators are needed for these plants. ARS scientists at Logan determined that the majority of desired wildflower species require bees for pollination, but some are satisfactorily pollinated with conventional pollinators (honey bees or alfalfa leafcutting bees). Researchers also identified two native pollinators that work well for some of the other flowers and have successfully developed methods to propagate these bees using shelters and nesting substrates. In addition to benefiting a small cadre of seed growers economically, this research will help restore degraded plant communities across millions of acres of western rangelands.

Appendix: Research Needs and Accomplishments Specific to Colony Collapse Disorder

Additional problems will be addressed as new technologies permit, or as new problems arise. For example:

> **Africanized honey bees** continue to expand their distribution in the southern United States. If genomics techniques allow, they will be used to develop additional means for identifying these invaders.
>
> **Colony collapse disorder** (CCD) is a devastating new problem. A **CCD Action Plan** was developed in 2007 by the Federal CCD Steering Committee (co-chaired by ARS and the Cooperative State Research, Education, and Extension Service) and is available online at www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf. A Progress Report on CCD-related research is due to be released in 2009. The following is a summary of ARS accomplishments on CCD to date:
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> The honey bee genome sequencing effort was led by ARS, in partnership with universities and industry (*Science*, 2007). It was the first insect to be sequenced since the *Drosophila* fruit fly, and its sequence has provided clues to bee mortality, including that by CCD. For instance, the bee genome was found to be lacking many of the detoxification and immunity genes found in *Drosophila*, perhaps explaining the honey bee's increased susceptibility to pesticides and pathogens, respectively. This is explained ecologically in the habitat and food of the honey bee (which feeds, naturally, on toxin-free nectar rather than plant tissues, which can be rich in toxins), as well as its sociality, on which it depends to rid the colony of diseased bees. Immunity may be key, since viruses have been implicated by ARS as highly suspect as one causal factor in CCD (*Science*, 2008).
>
> ARS has initiated an Areawide Program on Bee Health with the purpose of demonstrating that good bee management can maintain healthy bees despite the pressures of CCD. This program uses ARS technologies, such as bees developed with resistance to the varroa mite, the major bee pest (which also vectors bee viruses); special protein-rich diets developed to strengthen colonies for early spring build up; biorational miticides based on bee pheromones; and other treatments to mitigate disease and reduce stress on the colonies used in migratory beekeeping.
>
> A more detailed description of ARS accomplishments on CCD follows:
>
>> <u>2008: Honey bee viruses studied</u>. ARS scientists in Beltsville investigated the ranges and genetic traits of six honey bee viruses found in the United States. Significantly, scientists determined the past and present distributions of the Israeli acute paralysis virus (IAPV), a virus implicated in CCD. Other accomplishments include improving economical viral storage methods prior to diagnosis and adopting this protocol in analyzing field samples from the United States and abroad. Along with carrying out genetic analyses of frequencies of the major bee viruses, work continues to determine co-infection rates and implications for disease and, more fundamentally, to improve bee resistance to viruses.

Research on the viral causes of bee disease is aimed at decreasing the costs of beekeeping and assuring adequate pollination.

2008: Bee samples collected and preserved for diagnostic assays. Using field, microscopic, and genetic tests, Beltsville scientists have processed hundreds of bee samples (and hundreds of thousands of bees) in the past year, quantifying mites, bacteria, parasitic gut diseases, and viruses. In doing this, the unit has become a leader in developing efficient methods for collecting, preserving, and processing honey bees and hive materials for diagnostic assays. Information is shared freely with beekeepers, governmental regulators, and other researchers in an attempt to resolve the causes of CCD and prevent further losses for the beekeeping industry.

2007: Possible link established between IAPV and CCD. As published in *Science*, ARS scientists at Beltsville, in collaboration with researchers at several universities, established a link between a newly discovered virus, Israeli acute paralysis virus (IAPV), and bee colonies with colony collapse disorder (CCD). Of those colonies that suffered from CCD, nearly all had IAPV present, while healthy colonies generally did not have the virus. The origin of the virus is unknown at this time. ARS scientists at Beltsville also established that the virus has been present in the United States from 2002 or earlier (*American Bee Journal*, December 2007). Research suggests that IAPV could be contributing to the outbreak of CCD; however, more work is needed to substantiate that linkage. IAPV does appear to be a very good marker for CCD and its detection may aid in defining CCD.

2007: Diagnostic gene sets developed for CCD and other bee diseases. ARS scientists in Beltsville tested a broad panel of bee genes related to disease responses and stress responses against samples collected from CCD-implicated colonies and controls. Coupled with screens for 16 bee parasites and pathogens, this gene set helped identify the ‘sickest of the sick’ and provides a future diagnostic for colony health surveys.