

**United States Department of Agriculture  
Agricultural Research Service  
National Program 305**



**Crop Production  
FY 2023 Annual Report**

The Crop Production National Program (NP 305) supports research to develop knowledge, strategies, systems, and technologies that contribute to greater cropping efficiency, productivity, quality, marketability, and protection of annual, perennial, and nursery crops, and greenhouse and indoor farms, while increasing environmental quality and worker safety.

The Nation’s rural economic vitality depends on the ability of growers to profitably produce and market agricultural products including food, fiber, flowers, industrial products, feed, and fuels, while enhancing the natural resource base of crop production. Future financial success depends on increasing productivity, accessing new markets for specialized products, developing technologies to provide new opportunities for U.S. farmers, and utilizing tools and information to mitigate risks and enable rapid adjustments to changing market conditions. The farm sector has great and varied needs driven by a wide variety of resource, climatic, economic, and social factors that require an equally diverse array of solutions.

Contemporary cropping enterprises are complex and depend on highly integrated management components that address crop production and protection, resource management, mechanization, and automation. U.S. annual, perennial, and controlled environment crop production (e.g., greenhouse and other protected systems) are based on the successful integration of these components. The development of successful new production systems requires a focus on new and traditional crops; the availability and implementation of improved models and decision aids; cropping systems that are profitable and productive; production methods fostering conservation of natural resources; efficient and effective integrated control strategies for multiple pests and diseases; improved methods, principles, and systems for irrigation; improved mechanization and automation; and reduced inputs – all while sustaining or increasing yield and quality.

Production systems must better address the needs of small, intermediate, and large farming enterprises including those using field-, greenhouse-, indoor farm-, orchard-, and vineyard-based production platforms with conventional, organic, or controlled environment strategies. Additionally, adaptation and development of technologies are required to ensure a sustainable

and profitable environment for production agriculture. New technologies must address the need for lower cost, higher efficiency inputs that foster conservation of energy and natural resources, while maintaining profitability and promoting environmental sustainability. In addition, declining bee populations and honey production require special attention. Over the past several years, a myriad of pests and potentially adverse cultural and pest management practices have been threatening many of the bee species required for pollination of multitudinous crops. Colony Collapse Disorder (CCD) had increased honeybee (*Apis*) overwintering mortality to over 30 percent; and while CCD incidence has declined, bee mortality remains unsustainably high. Also, as new crops or niches are introduced, there is an increasing need for non-honeybee pollinators for specific crops or protected environments.

National Program 305 coordinates and collaborates extensively with other ARS National Programs, universities, and industries in adapting and incorporating technologies, approaches, and strategies that enable the advancement of the Nation's agricultural industry and enhanced international competitiveness.

**This National Program is divided into two main research components:**

**Component 1.** Integrated Sustainable Crop Production Systems

**Component 2.** Bees and Pollination

Below are National Program 305 accomplishments from fiscal year 2023, grouped by research component. This report is not intended to be a progress report describing all ongoing research, but rather an overview that highlights accomplishments, some of which are based on multiple years of research (not all research projects will reach an “accomplishment” endpoint each year).

**Component 1. Integrated Sustainable Crop Production Systems**

**Spotted lanternfly threatens specialty crops.** The spotted lanternfly (SLF) is an invasive pest in the United States that feeds on more than 103 different plant species, but more information is needed about its potential threat to specialty crops and forest species. ARS scientists in Kearneysville, West Virginia, and collaborators conducted laboratory and field trials that expanded available information about SLF host plants. They found wine grapes (*Vitis vinifera*) is a preferred host in the field and supports strong development and survivorship, while cultivated apple and peach do not support SLF development. Among wild hosts, black walnut, riverbank grape, and invasive tree of heaven all support strong development and survivorship. These results indicate SLF poses a threat to the winegrape industry, and wild hosts such as black walnut, riverbank grape and tree of heaven can likely support its establishment in new regions. This information will help growers develop effective management strategies for this emerging and invasive insect pest.

**Virtual Grower energy modeling update and beta launch.** ARS researchers in Wooster, Ohio, developed Virtual Grower, a decision-support software tool for designing a virtual greenhouse, estimating corresponding energy usages for heating and lighting, and evaluating various energy-

savings options. These researchers recently updated Virtual Grower to version 4, which includes improved energy and plant growth models that more accurately estimate greenhouse energy usage, and its conversion to an online platform offers more accessibility. The beta test website was launched in May 2023. Virtual Grower is the only software tool available to growers and researchers that specifically targets modeling greenhouse crop scheduling and energy consumption. The beta is being tested by growers in greenhouse management classes and selected research colleagues. These ongoing efforts improve energy efficiency, lower overall energy use, and train students and growers in greenhouse management.

**Silicon induces plant defensins to protect against plant stresses.** Plant stress due to pests, diseases, or adverse environmental conditions can negatively impact plant growth and yield. Silicon can help plants tolerate stresses such as chilling injury, heavy metal toxicity, and powdery mildew disease outbreaks, but the molecular mechanisms underlying this beneficial response are not well understood. ARS researchers in Wooster, Ohio, and University of Toledo collaborators identified histidine-rich defensins, which are part of the immune system, that can be upregulated by silicon. These defensins are also involved in reducing disease severity, which shows one potential pathway silicon can use to confer disease suppression in plants. This novel discovery helps researchers further understand how silicon functions in plants and identified key targets plant breeders can use to increase crop resilience to pathogens.

**Winter oilseed crops reduce early summer weed growth.** Using winter oilseeds as cash cover crops is gaining popularity. A previous study indicates that certain winter mustard oilseed species with good freeze-hardiness can suppress early summer weeds, including herbicide resistant weed species. ARS researchers in Morris, Minnesota, and Fargo, North Dakota, conducted a study at both locations to determine winter survival and weed suppression traits in winter camelina and several winter canola lines selected for improved winter survival. Winter camelina had much greater winter survival than canola, and the following spring, camelina suppressed weed biomass by more than 90 percent at both locations. Winter-hardy canola lines were identified that breeders can use to improve freeze tolerance in commercial varieties. These findings benefit crop breeders and geneticists looking to improve crop freeze-hardiness and farmers and researchers looking for cropping strategies to improve integrated weed management.

**Optimizing UAV spray patterns for pest management.** Agriculture is rapidly embracing the use of unmanned aerial vehicles (UAVs) for applying agrochemical products to manage pests. However, there is a significant gap in the data related to spray pattern uniformity and droplet distribution that is essential to guiding best management practices, and understanding these factors is critical to sustainable and effective UAV use. ARS researchers in College Station, Texas, used four different commercial UAV platforms to study how application height and ground speed affect these parameters and found that each UAV platform could produce an acceptable spray pattern irrespective of payload capacity, and that an increase in payload widened the effective swath. These findings provide valuable guidance for aerial applicators using UAVs effectively in pest management and will facilitate developing efficient pest management strategies to enhance the sustainable use of UAVs in agriculture.

**Air-pinch valve to improve hollow-cone nozzles for variable-rate sprayers.** Pesticide sprayers are commonly used for efficient and effective crop protection, and electric pulse width modulation solenoid valves are the critical sprayer components that provide precision variable rate applications. However, these valves cannot shut off completely if sprayers are not rinsed thoroughly after applying adhesive additives or physically incompatible powder pesticides. ARS researchers in Wooster, Ohio, investigated an air-pinch valve that separated the chemical liquid from the valve chamber as an alternative to the electric valve. They discovered the air-pinch and electric valves were equally accurate in flow rate modulations for hollow-cone nozzles operated at different pressures. Similarly, droplet size distributions and classifications from the hollow-cone nozzles regulated with both valves were also comparable across the flow rate modulation ranges. These findings indicate the air-pinch valve could be an alternative to conventional electric valves and is being recommended to farm equipment companies to improve the accuracy and reliability of their precision variable rate sprayers.

**Reducing off-target damage from synthetic auxin herbicides.** The global use of synthetic auxin herbicides is frequently linked with unintended damage to non-target crops and pollinators, disrupting effective weed management. ARS researchers in College Station, Texas, and academic colleagues conducted field trials to measure the downwind movement and damage of these herbicides. They assessed how different application techniques and weather conditions affect spray drift and deposition, with a specific focus on soybean impacts. Results revealed that aerial applications doubled the downwind deposition and plant damage compared to ground applications. However, offsetting aerial spray passes several swaths in the upwind direction significantly reduced both deposition and plant damage. These findings provide valuable guidance for better herbicide management practices and demonstrate that specific application adjustments can significantly reduce drift and associated crop damage. This research offers vital insights to ARS partners and stakeholders and will facilitate mitigating the negative impacts of auxin herbicides on non-target crops and pollinators, thus contributing to more sustainable agricultural practices and broader environmental protection.

**Improving pennycress production management.** Pennycress is a new winter oilseed crop that can be used for biofuels and bioproducts. This species can be planted in fall after corn, soybean, or wheat and relay-cropped the following summer with soybean, thereby producing two crops on the same field in a single year. ARS researchers in Morris, Minnesota, demonstrated that using careful soybean variety selection and proper planting schedules can maximize soybean yields without lowering pennycress yields. The researchers and university collaborators showed that pennycress can be directly combine-harvested versus swathing because pennycress seeds mature rapidly. Results benefit farmers interested in producing pennycress and relay cropping, university extension specialists, ag consultants, industry partners, and other scientists seeking ways to improve pennycress production.

**New fertilization, irrigation, and compost methods reduce greenhouse gas emissions and maintain crop yields.** ARS researchers in Davis, California, and researchers at the University of California, Davis, completed a global structured review of how cover crops affect soil biological, physical, and chemical attributes known as soil health indicators. Given the priority of both USDA and various California state agencies to support Climate Smart Agriculture, they examined the

benefits of cover crops for soil health, crop productivity, and economic and ecological factors. They found cover crops were associated with higher levels of soil organic carbon—a prized soil health indicator—and soil texture class influenced the magnitude/frequency of this and other soil health indicators. This work demonstrates that in nearly all soil texture classes, cover crops tend to be associated with most soil health indicators, regardless of cover crop type, cropping system, and world region. However, cover crop use did not consistently reduce soil compaction across all soil textures, suggesting other mitigating practices for soil compaction are needed in combination with cover crops. These findings suggest that cover crops improve and/or sustain soil health indicators, which may protect soils for crop production and promote Climate Smart Agriculture.

**Identifying weeds in sugarcane fields with remote imagery.** Controlling weeds is critical for profitable sugarcane production systems. ARS scientists in Houma, Louisiana, and cooperators from Louisiana State University and Texas A&M University detected weeds in sugarcane fields using leaf reflectance measurements and pigment analyses. Leaf samples were collected from four commercial Louisiana sugarcane varieties and from nine weed species commonly found in sugarcane fields. In all cases, leaf reflectance data successfully differentiated sugarcane from weeds. The accuracy of the classification varied from 67 percent to 100 percent for individual sugarcane varieties and weed species, and sugarcane was never misclassified as a weed. The successful implementation of this technology as either an airborne system to scout and map weeds or a tractor-based system to identify and spray weeds in real time would offer sugarcane growers a valuable tool to manage their crops. Accurately targeting weeds could reduce total herbicide applications, save growers costs, and reduce environmental impacts.

**Modified row spacing improves soybean yield in winter camelina/soybean relay cropping system.** ARS researchers in Morris, Minnesota, pioneered the development of relay cropping soybean with winter camelina and pennycress. While much is known about biological nitrogen fixation (BNF) in traditional soybean production systems, very little is known about soybean BNF when relay-cropped with camelina. ARS researchers in Morris, Minnesota, showed that nitrogen-fixing bacteria successfully fixed nitrogen in relayed soybean roots. However, competition between camelina and soybean during the relay phase, when soybeans are growing between camelina rows, resulted in lower nitrogen fixation and lower grain yield in relayed production, compared to conventionally grown soybean. Findings from this research suggest that modifying plant spacing to reduce plant-to-plant competition during the relay phase may boost BNF and seed yields in relayed soybean. Results benefit researchers exploring ways to improve productivity in relay cropping systems, as well as farmers, ag consultants, and extension specialists who are beginning to implement this new cropping system.

**Water footprint established for blueberries.** A “water footprint” is defined as the amount of water needed to produce a unit of a particular product and measures freshwater resource use for human activities, including agriculture. ARS researchers in Corvallis, Oregon, collaborated with the University of Buenos Aires (Argentina) and the University of Concepción (Chile) and determined the water footprint for three widely grown blueberry cultivars: ‘Star’, ‘Emerald’, and ‘Snowchaser’. The annual footprint of each, which included water utilized from rain, drip irrigation, and sprinklers for frost protection, differed among the cultivars. Star used from 25 to

69 gallons of water to produce a pound of berries, Emerald used from 35 to 118 gallons of water to produce a pound of berries, and Snowchaser used from 64 to 487 gallons of water to produce a pound of berries. Snowchaser bloomed at the beginning of winter and required the most water for frost protection, while Star lost most of its leaves during the winter, flowered late, and consequently used the least amount of water. Irrigation designers can use this information to quantify water requirements for each cultivar and allocate water for irrigation and frost protection accordingly.

**Refining corn nitrogen fertilizer recommendation tool.** Nitrogen (N) fertilizer is a major expense for corn producers, and overuse is both uneconomical and leads to air and water contamination. In Minnesota, N fertilizer rates for corn production are mainly determined using an online calculator available for the Corn Belt Region, which uses N fertilizer cost, corn grain price, and crop rotation to calculate recommended N application rates. ARS researchers in Morris, Minnesota, found that including weather, soil types, and corn hybrid (early vs. late) improved online estimates of N requirements for corn production in Minnesota. These results provide needed information for extension specialists, consultants and producers wanting to assess how current N fertilizer costs and corn grain prices affect the net benefits of using N fertilizer for corn production.

**Identifying ideal sampling times to track grapevine water stress with hyperspectral tools.** Grape growers need new methods to accurately track vine-water stress for optimizing deficit-irrigation strategies that maximize fruit quality and yield. Current methods are labor intensive and typically assess only a few vines in a vineyard. Indices from hyperspectral reflectance measurements (HI) could inform management across larger acreage, while still providing resolution for individual vines, but require refinement to be a viable tool in commercial vineyards. ARS researchers in Davis, California, and other locations in collaboration with researchers from University of California, Davis, and industry, found that vine water-stress was best detected during morning and late afternoon. This suggests that satellite and drone overpasses, which typically occur during midday, may miss the ideal times to detect water stress. Industry partners have altered measurement timings in response to these results.

**Scent from a parasitic fungus attracts aphid herbivores.** The behavioral response of the green peach aphid was characterized in response to the scent emitted from a parasitic fungus (*Beauveria bassiana*) that infects and kills its insect host. ARS researchers in Wooster, Ohio, demonstrated that volatiles emitted from *Beauveria bassiana* attract rather than repel aphids. Attraction to the parasitic fungus will result in increased infection since direct contact is required. These results will aid researchers in developing an attract-and-kill strategy using parasitic, entomopathogenic fungi as microbial control agents to reduce the reliance on conventional insecticides.

**An effective biopesticide for *Sclerotinia sclerotiorum*.** *Sclerotinia sclerotiorum* is a serious issue in high tunnel tomato production and causes important disease of multiple crops in the north central United States. Control methods based on natural host resistance or fungicides have not provided sufficient control to date. Fungal viruses that infect *S. sclerotiorum* and weaken the ability of the fungus to grow or to infect plants could contribute to control of the disease. ARS

researchers in Wooster, Ohio, developed an effective biopesticide from crude viral particle preparation as fungicide spray. Growers can also adapt the technology to simultaneously control *Botrytis cinerea* in the greenhouse because mycovirus also reduces the pathogenicity of other Sclerotiniaceae fungi.

**New tool to track a hidden tomato disease that can reduce yield by 50 percent.** Tomato corky root rot is a root disease that can half tomato yield and is found in 50 percent of tomato high tunnels. This disease is often undetected until it is too late because it attacks roots, is slow growing, and difficult to isolate from plants, so improved detection methods are needed. ARS researchers in Wooster, Ohio, developed a quantitative polymerase assay (qPCR) to rapidly detect corky root rot pathogens in roots and soils. This assay provides a tool for researchers to rapidly detect and track the pathogens to provide farmers with strategies to better manage this disease.

## Component 2. Bees and Pollination

**Effects of nutritional deprivation on honey bees.** Honey bee colonies are routinely provided with nutritional supplements during periods of drought and associated food shortages. In a changing climate, these events may increase in intensity and frequency, or occur at unexpected intervals, and understanding how bees respond to challenging nutritional times is critical for determining management strategies and breeding tactics. ARS researchers in Baton Rouge, Louisiana, found that when colonies were deprived of pollen, they became generally more aggressive than colonies that had access to pollen resources. Temperament and aggression are sometimes used to select breeding stock, so these findings can help breeders enhance traits favorable to changing climates. Additional research found that honey bee stocks derived from Italian and Russian honey bees respond to nutritional deprivation in distinct ways. Russian honey bee workers appear to conserve individual nutritional stores at the expense of producing nutrient rich brood food components, but Italian honey bee workers continue production of brood food components at the expense of nutrient stores. This has potential implications for traits that are beneficial in a changing climate and informs beekeepers about when and how to use nutritional supplements based on the type of honey bee.

**Artificial intelligence can diagnose brood disease.** Honey bee brood disease causes significant colony loss in the United States, but diagnosis based on larval symptomology is subjective and often depends on context. Antibiotics are typically used prophylactically to treat bacterial diseases such as European Foulbrood, but this breeds molecular resistance, causes dysbiosis of the gut microbiome, and often fails to treat the underlying illness. ARS researchers in Tucson, Arizona, are using machine learning to modernize brood disease diagnosis. Based on samples and corresponding images of diseased larvae collected throughout the United States, they recovered a variety of known and unknown disease-associated bacteria and virus from samples diagnosed as known disease or idiopathic. Preliminary machine learning algorithms using only image symptomology were successful in predicting microbiome results. This resource enables the early diagnosis of brood disease based on a smart phone photograph, and represents a valuable resource for beekeepers, apiary inspectors, and scientists.

**Using advanced technologies to improve honey bee breeding.** The Russian honey bee stock was the first population developed using a marker-based, genetic stock identification (GSI) assay. Recent developments in sequencing have improved the understanding, evaluation, and use of genetic tools to advance breeding efforts, and an updated stock identification panel using novel genetic variation would provide a more accurate discrimination tool and serve as a pilot for future marker panels. ARS scientists in Baton Rouge, Louisiana, used recent genomic data and historically preserved DNA to develop a new marker panel using a microfluidic platform. The resulting assay outperformed the original GSI while simultaneously increasing efficiency and reducing cost. This novel approach is currently being finalized to incorporate in stakeholder breeding decisions. In addition, the method is inherently modular and has pioneered analytical approaches that can be directly implemented in trait-based or other marker-assisted selection panels.

**Honey bee genetics and winter survivorship.** Challenges to honey bee health arise from disease agents, chemical stress, and nutritional challenges. Bees are especially stressed in the winter due to temperature, parasites, and the inability to replace aging worker bees and queens. ARS scientists in Beltsville, Maryland, discovered a key honey bee cell signaling pathway, and then used a therapeutic strategy to target this pathway, improving bee and colony survivorship. They also showed how bees differ in their susceptibilities to chemical stress in winter, improving survivorship management options. This work was coupled with a nationwide analysis of honey bee varieties and helps advance understanding climate-based preferences of honey bee stock. ARS scientists in Beltsville are also leading the Beenome100 project (which includes multiple outgoing agreements and the coordination of 20 research laboratories), which is focused on producing reference genomes for 100+ U.S. bee pollinators and leveraging these genomes for bee health. For instance, genetic comparisons of diapause and overwintering between honey bee and hundreds of other bee species used in pollination can help improve winter survival in honey bee. This effort is paying off with insights on bee host use and climate expansion and improved methodologies for describing bee genomes.

**Honey bee foragers prefer nutrient-rich mixed-species pollen.** Honeybees providing pollination services are regularly exposed to a monoculture diet. ARS researchers in Davis, California, showed that when given a choice, bees prefer pollen from intercrops that contained a mix of species. Analyses of pollen from almond orchards, sunflower fields and from mixed-species intercrops showed that mixed-species pollen is rich in important dietary compounds that support healthy honey bees. This result supports the need for pollinator habitats and intercrop plantings in almond orchards and other agricultural fields.

**Western bumble bee decline linked to climate change and pesticides use.** The historically abundant western bumble bee was developed commercially to deliver key pollination services to diverse cropping systems, especially greenhouse tomatoes. However, a pathogen outbreak in these western bumble bee populations halted their production indefinitely. ARS researchers in Logan, Utah, and collaborators found that western bumble bee population declines are also



linked to increasing drought conditions and pesticide use in the western United States. These results will support the U.S. Fish and Wildlife Service and state agencies in making informed decisions on managing western bumble bee habitat.

**Amending spray solutions to increase pesticide efficiency and reduce runoff.** The effectiveness of pesticide applications in crop systems can be increased significantly by increasing droplet retention and spread, which is influenced by the interaction between the adhesive characteristics of the droplets and leaf surface structures. ARS researchers in Wooster, Ohio, investigated spray droplet retention and spread capabilities on leaves by changing physical properties of spray solutions with different types of adjuvants. They found the organo-silicone adjuvant provided the best droplet retention on the rough and difficult-to-wet leaves at concentrations of 0.75 percent or higher. The polymer-based adjuvant improved droplet retention on easy-to-wet leaves but was ineffective on the difficult-to-wet leaves. The information from this research provided key components for chemical formulators and growers to select or formulate optimal adjuvants to increase chemical retention on plants.

**Cold storage evaluated as part of a Varroa management strategy.** Varroa mites feed on developing bees and transmit disease, making them one of the most serious problems in beekeeping. Placing honey bee colonies in cold storage has been proposed as a way to induce a pause in brood production and produce bee colonies with low Varroa mite levels in the spring. ARS researchers in Tucson, Arizona, conducted a 2-year study that exposed colonies to an October cold storage period and/or a subsequent miticide application. They found the full treatment did not reduce spring Varroa levels, but cold storage did stop brood production. Long-term impacts of cold storage on adult bee populations, colony thermoregulation, and stress biomarkers were low compared to nutritional effects resulting from yearly differences in bee forage availability. Although further work is needed to optimize treatment schedules in the late summer and fall, cold storage may be an effective way to reduce brood as part of a Varroa management plan.

**Controlling Varroa mite impacts on honey bees.** With external and ARS support, ARS scientists in Beltsville, Maryland, led research testing candidates for the chemical control of Varroa mites, work that led to CRADA agreements and pending inventions. Additional efforts have led to the identification of cues used by Varroa to locate host larvae and two papers related to the pathways by which mites transmit viruses to bees. Finally, ongoing research has identified plant compounds and amino acids that help reduce gut parasites and mite-transmitted viruses, respectively.

**The origins of bees and how they came to dominate pollinators.** Bees are the most important and effective pollinators of flowering plants. This interaction began about 120 million years ago, but uncertainty of how and when bees spread across the planet has greatly obscured investigations of this key partnership. ARS researchers in Logan, Utah, and an international group of researchers used extensive genomic and fossil evidence to conduct a new analysis of bee biogeography. They confirmed that bees originated in Western Gondwana (South America + Africa) about 124 million years ago and estimated a history for how bees subsequently colonized other terrestrial habitats and diversified in association with flowering plants. This

information will be essential to understanding how different flowering species are linked to specific groups of bees, which is critical for conserving those plants and their pollinators.

**Propolis is a potent prebiotic in the worker bee gut and colony.** Yeast overgrowth in the gut is problematic in host-microbial systems, including humans. ARS researchers in Tucson, Arizona, tested the effect of plant resins (propolis) that worker bees applied on fungi throughout the colony and found that propolis enrichment radically restructures the bacterial-fungal relationship throughout the gut and hive. Collective gut fungi, primarily yeasts, were reduced 10-fold, bacterial diversity decreased significantly, host immune gene expression was collectively reduced, and beneficial probiotic species increased dramatically in both the worker gut (3-fold) and throughout the hive environment (10-fold). These findings about the positive impact of propolis processing on immunity, disease, and microbiota give beekeepers informed information about the economic viability associated with installing propolis traps within their hives.

**A high-quality genome assembly of the endangered rusty patched bumble bee.** ARS researchers in Logan, Utah developed the first high quality genome assembly of the endangered rusty patch bumble bee, which is the first bee in the continental United States protected under the U.S. Endangered Species Act. In addition to sequencing thousands of rusty patch bumble bee genes, ARS researchers detected genetic sequences associated with a significant bee pathogen, *Varimorpha bombi*. The pathogen causes dysentery, reduces mating success, and increases the chances of premature death in overwintering bumble bee queens. The results of this study will support U.S. Fish and Wildlife Service and state agencies in making informed decisions on rusty patched bumble bee breeding and management programs.

**Spray adjuvants negatively affect honey bee reproduction and health.** Honey bee colonies depend upon a healthy egg-laying queen for colony survival and growth to meet critical pollination needs. In recent years, beekeepers have found that queen survival and performance has greatly declined. While organosilicone spray adjuvants are commonly added to tank mixes of pesticides and herbicides, ARS researchers at Davis, California; Logan, Utah; and Baton Rouge, Louisiana, found that these adjuvants negatively impact the egg laying abilities of queen honey bees. In addition, workers fed the organosilicone spray adjuvants had higher levels of deformed wing virus. These results support other findings that suggest these chemicals are of concern for bee health. ARS shared this information with the Environmental Protection Agency, the Almond Board, and other grower groups through the Honey Bee Health Coalition, to promote the use of other adjuvants that do not have these impacts and promote greater honey bee health.

**New antiviral drug target identified and field-tested in honey bees.** Honey bees contend with many parasites and pathogens that greatly impact colony productivity and survival. Viral infections, either transmitted by the parasitic *Varroa* mite or spread from bee to bee, cause both direct and subtle effects on bee behavior and lifespan. Despite the fact that honey bees are often co-infected with multiple viruses at both the individual bee and colony levels, there are no antivirals available for beekeepers. ARS researchers collaborated with Louisiana State University partners on lab and field tests of a compound that activates honey bee potassium

ion channels. By regulating these channels, the drug increases a honey bee's ability to fight infection of multiple viruses. Field tests of this drug in virus-inoculated colonies confirmed laboratory results and for the first time showed that pharmacological treatment against viruses is possible for honey bee colonies. This research identified a physiological target to focus on in honey bees that can be used to develop a drug treatment that could be approved to improve honey bee resilience to viral infection. As there are no treatments currently available against honey bee viruses, finding ways to reduce the impacts of virus in honey bees is a critical need to reduce potential virus spread to other bee species and increase the sustainability of the beekeeping industry.

**Genetic mutation and beekeeper management predict mite resistance to miticide.** The parasitic Varroa mite significantly impacts honey bee health and is the largest driver of colony mortality. Recent reports indicate that Amitraz, the most widely used chemical treatment for mite control, has provided inconsistent results. How often and why the mites are developing resistance is key to improve treatment decisions. ARS sampled mites from more than 1,382 colonies from 146 apiaries across 82 beekeeping operations and found each beekeeping operation has its own resident Varroa population with Amitraz resistance levels likely to be driven by amitraz use patterns. Highly resistant Varroa tends to be the result of overuse (intensity and frequency) and overreliance on Amitraz as the sole measure of Varroa control. Evidence also indicates that Amitraz-resistant Varroa do not move passively among beekeeping operations, even in close proximity and at high colony density. Through an international effort, a genetic mutation strongly associated with amitraz resistance in Varroa in the United States has been confirmed. This research is important to the beekeeping industry because the reduced efficacy of amitraz to control Varroa is increasing demand for alternative miticides and Varroa management practices. Results have also informed beekeepers of the need to change management practices by rotating types of miticide treatment to reduce the likelihood of resistance developing in their population.

**How honey bee colony nutrients change over time.** Natural and supplemental diets available to hives throughout the year must meet the colony's nutritional needs, as poor nutrition compromises colony health. ARS researchers in Maricopa, Arizona, characterized the nutrients that were stored and utilized by the bee during key seasonal changes in the colony and conducted field trials with colonies in the U.S. Upper Midwest to test the adequacy of fall commercial pollen supplements. For colonies in both the Upper Midwest and Tucson, macronutrients like total lipid and protein correlate with seasonal patterns of hive growth and contraction. The direction and magnitude of these changes are similar, but their timing differs. These results suggest that lipid storage is key for colony health and survival through the winter, so colonies should be fed more lipids in the fall than are typically available in commercial diets. Colonies that are expanding in the spring need more protein, so they should be fed diets high in protein, which is typical of most commercial diet supplements.

**A century of sampling reveals declining wild bee diversity.** Over the past few decades there has been increasing concern about the decline of wild bees around the world and this decline is often associated with habitat loss. To test if species losses are also occurring in preserved habitats, an ARS scientist in Logan, Utah, led a team studying wild bee community change in a

long-term protected nature preserve. Using museum records of bees at the nature preserve since 1921 and recent sampling efforts by the research team, they found evidence of significant community change over time, and a significant recent decline in species richness and abundance. Species losses were often associated with certain traits such as diet specialization and nesting strategy, as well as larger body size. They also found that species with more southerly ranges did well at the preserve over this period. This study provides evidence that wild bee losses are occurring in protected habitats, that certain bee traits may put species at higher risk of decline, and that community change may be driven by more widespread impacts such as climate change. It is critical to understand which pollinators may be in danger of disappearing because of factors such as climate change in order to conserve natural areas and their plant species. For growers, this information may be critical in protecting the pollinators for their crops.

**Poor air quality linked to lowered immune system functions in honey bees.** Higher daily maximum temperatures in central California are related to poorer air quality. Honey bees, essential for crop pollination, are likely to be impacted. ARS researchers in Davis, California, and university collaborators reported that poor air quality was associated low expression of honey bees' genes tied to immune system strength and high expression level of genes tied to oxidative stress. There was a negative correlation between Varroa mite loads and heat shock protein gene expression, suggesting that mite-infested colonies may have a limited ability to buffer against extreme temperatures. This finding provides insights into aspects of honey bee health that may be directly compromised with environmental stresses related to climate change.

**Alfalfa leaf-cutting bees can regulate the developmental fate of their progeny.** In response to climate change, bees may be able to adjust their development and responses via a type of hibernation or diapause, which is needed to survive the winter. How this is regulated had remained a mystery. ARS researchers in Logan, Utah, and their collaborators found evidence that female bees can influence the development and diapause of their progeny via mRNA transcripts that they incorporate into the eggs. The transcripts are altered in response by the female bee to changing seasonal conditions and not diet. These research findings will help enable more accurate predictions on how climate change will impact bee development and influence the availability of important bee pollinators in the upcoming year. This information will be critical for bee managers and alfalfa seed growers who depend upon these bees for the successful pollination of their crops.

**The health of pollinators, both wild and managed.** The health of pollinators, both wild and managed, is of national and international concern. In 2020, a new ARS research unit focused on pollinator health was established in Stoneville, Mississippi, and most of the scientific and support positions have been filled. ARS researchers in Stoneville are conducting new research projects focusing on pollinator health in southern agroecosystems and examining the interactions between bees, agricultural production, and the surrounding landscape.

**Bees visit a wide diversity of flowers during blueberry pollination.** Commercial blueberry production is dependent on managed bees for pollination and development of marketable

fruit. Growers rent honey bee colonies and purchase managed bumble bee colonies for pollination. However, blueberry pollen is low in protein, and beekeepers are concerned that their colonies may suffer poor health outcomes due to poor nutrition while pollinating blueberries. An ARS scientist in Logan, Utah, led a team that collected and identified bee-collected pollen from 84 honey bee colonies and 120 common Eastern bumble bee colonies as the bees pollinated commercial blueberry crops in Michigan. Both bee species collected pollen from a wide diversity of plants; honey bees collected from 21 plants and bumble bees collected from 29 plants, while blueberry pollen was found in less than 30 percent of the pollen brought back to any colony. Given the diversity of pollen collected by managed bees, it is unlikely that colonies are nutritionally deprived in these landscapes and suggests there may be other causes of poor colony performance, such as diseases, parasites, or pesticide exposure. Understanding factors impacting the pollinators is critical in ensuring their performance; in the case of blueberry fields in Michigan, nutrition can be ruled out as a factor and this study provides critical findings to both growers and beekeepers.

**Molecular data from museum specimens reveals declining genetic diversity in the western bumble bee.** *Bombus occidentalis* is an important bumble bee species that has declined in abundance across its range in the western United States since the mid-1990s. The species is currently being considered for listing under the Endangered Species Act. Updated population genetic analyses to assess the genetic resiliency of the species, and its close relative *B. mckayi*, are needed to inform conservation decisions. ARS researchers in Logan, Utah, and collaborators at Utah State University, Ohio State University, the U.S. Fish and Wildlife Services, and other agencies used museum specimens collected between 1960 and 2020 to examine genetic structure and diversity in populations of *B. mckayi* and *B. occidentalis*. They found evidence of declining genetic diversity through time in both *B. occidentalis* and *B. mckayi*, with evidence of decline predating abundance-based measurements. Although both species still have significant genetic diversity, declines in genetic diversity through time may make recovery more challenging. This work will help inform conservation decisions for the western bumble bee and demonstrates the value of museum specimens to population genetics and conservation research.

**Development of new genetic markers for the blue orchard bee.** ARS researchers in Logan, Utah, developed 22 new genetic markers to determine the genetic health of the blue orchard bee, which is an important managed pollinator of U.S. orchard crops. The new genetic markers capture important genetic information that may be used to make informed breeding and management decisions about the blue orchard bee, which is increasingly used for commercial pollination of fruit and nut crops. This information will be critical to bee managers and growers and ensure the genetic diversity of the bees and their overall performance.

**Discovery of critical lifecycle and biological traits enables control of a highly destructive bee parasitoid.** Bees have many different predators and parasitoids that can affect their populations and in turn impact the successful pollination of seed crops. The alfalfa leaf-cutting bee is widely used for pollinating alfalfa seed crops and canola, but the tiny parasitic wasp *Melittobia* can reproduce extremely quickly and wipe out entire stores of bees and disrupt crop pollination. ARS researchers in Logan, Utah, completely determined how the wasp lives

and develops in relation to the bees and developed methods to kill the wasp before it can cause damage. This information has been shared with alfalfa seed producers who depend upon these bees for successful pollination of their crops. These methods may also be applied to other species of bees that can be parasitized, protecting many species of pollinators used for commercial nut, fruit, and seed crop pollination.

**Starvation-associated volatiles from adult honey bee workers serve as attractants to nest workers.** The palatability and subsequent consumption of supplemental bee nutrition, which is provided in critical times of forage dearth, can vary greatly. Adult honey bees deprived of food resources solicit food donation from other workers, who in turn provide nutrients in the form of glandular secretions. ARS researchers in Tucson, Arizona, identified odors that attract workers (e.g., methyl benzoate) and mediate trophallactic exchanges among food donors and receivers within the nest. Identifying odorants mediating such trophallactic exchanges helps explain feeding interactions of nest bees with other castes/life stages and provides a mechanism beekeepers can use to make supplemental nutrition more palatable.

**Development of a cryopreservation protocol for insect conservation.** Insect populations are declining worldwide, and germplasm cryopreservation is a vital tool for conserving both economically and ecologically important species. Traditionally, germplasm cryopreservation requires the development of species-specific protocols and the use of readily available liquid nitrogen, which greatly inhibits germplasm collection at remote sites. ARS researchers in Fargo, North Dakota, developed a medium for the cryopreservation of honey bee sperm that enables collection and transportation at ambient temperatures. The germplasm can be at ambient temperatures for up to 6 months before the samples need to be placed into liquid nitrogen for long-term storage. This novel cryopreservation protocol provides conservation organizations with a new tool for preserving the world's insect biodiversity.

**Cryopreservation of sperm from recently deceased insects.** There is an alarming worldwide decrease in populations of economically and ecologically important insect species, and new conservation technologies are urgently needed. ARS researchers in Fargo, North Dakota, demonstrated that viable male insect germplasm can be collected up to 24 hours after the death of an individual, cryopreserved in liquid nitrogen, and later revived. Originally developed for the honey bee, this technology has been extended to a native bumble bee species and the monarch butterfly. This new technology offers conservation organizations a means to maximize the genetic potential of each male within a breeding colony.

**Antibiotic treatment for Nosema prior to cold storage overwintering can increase colonies for almond pollination.** Nosema is a gut parasite of honey bees that can reduce overwintering colony size and survival and limit the number of colonies available for almond pollination. ARS scientists in Tucson, Arizona, conducted a study to determine how diet and antibiotic treatment affect Nosema infections in colonies overwintered in cold storage and used for almond pollination. Colonies were fed diets of pollen or commercial protein supplement prior to overwintering. Colonies fed each diet were divided into groups that were either treated with antibiotics or were left untreated. Nosema levels in colonies fed either diet was significantly higher in untreated compared with treated colonies, and 40-55 percent of the

untreated colonies had infection levels that reduced colony size and survival after overwintering. The results indicate that diet alone cannot mitigate the effects of *Nosema* infections, and beekeepers should treat for *Nosema* prior to cold storage overwintering or risk having fewer colonies to rent for almond pollination.

**Removal of Varroa mites from whole honey bee colonies by powdered sugar shake dusting.**

Varroa mites are susceptible to control by dusting treatments that cause mites to lose their grip on their adult worker hosts. ARS researchers in Tucson, Arizona, developed novel powdered sugar dusting techniques that provided a fast, effective, and low impact treatment for mite removal from whole bee colonies. Phoretic mites were removed by shaking a mass of worker bees into a screen box, dusting them with powdered sugar, then shaking the honey bees to dislodge the mites. This powdered sugar shake method removed 92 percent of phoretic mites from worker honey bees in three weekly applications, a control rate comparable to commercial miticide treatments without chemical residues from the miticides. Powdered sugar applications had no deleterious effects on brood rearing, adult populations, or queens, and as a non-toxic agent, can be used during honey flows. This method removes the mites more rapidly from the honey bees compared to chemical treatments but is labor intensive.

**Juvenile hormone benefits honey bee reproduction.** Exposing queen honey bees to field-relevant doses of insect growth disruptors through worker diets does not negatively affect the queen's reproductive output or the health and behavior of her worker offspring. However, ARS researchers in Albany, California, observed a positive effect on egg hatching rates and queen acceptance behaviors in the offspring of queens exposed to a juvenile hormone mimic, suggesting juvenile hormone may have a beneficial effect on honey bee queen fecundity. This finding may lead to the development of a novel treatment beekeepers could use to enhance queens' longevity, honey bee colony stability, and healthy hives.

ARS welcomes your input regarding our ongoing research programs. If you have any questions, please do not hesitate to contact the National Program 305 team: Kevin Hackett ([kevin.hackett@usda.gov](mailto:kevin.hackett@usda.gov)), Joseph Munyaneza ([joseph.munyaneza@usda.gov](mailto:joseph.munyaneza@usda.gov)), Jack Okamuro ([jack.okamuro@usda.gov](mailto:jack.okamuro@usda.gov)), Timothy Rinehart ([tim.rinehart@usda.gov](mailto:tim.rinehart@usda.gov)), Roy Scott ([roy.scott@usda.gov](mailto:roy.scott@usda.gov)), Steve Young ([stephen.young@usda.gov](mailto:stephen.young@usda.gov))