The Crop Protection and Quarantine National Program (NP 304) addresses high priority insect, mite, and weed pest problems of crops, forests, urban trees, rangelands, postharvest systems (such as stored grains), and natural areas.

U.S. agriculture provides the Nation with abundant, high quality, and reasonably priced food and fiber. From corn and cotton, potatoes, peanuts, pumpkins, and peas to apples, alfalfa, almonds, soybeans, citrus, nuts, berries, and beans, American agriculture annually plants over a quarter of a billion acres of food and fiber crops worth over $115 billion. Additionally, agricultural commodities represent about six percent of the total value of all our domestic exports. Economic losses of our food and fiber due to insects, mites, and weeds, however, are considerable, with estimates in the tens of billions of dollars. Pest control includes cultural, biological, physical, and chemical methods. Non-chemical methods based on biological knowledge continue to expand, but the Nation continues to depend heavily on chemical control to produce agricultural commodities. Maintenance of our arsenal of valuable agricultural chemicals is a constant challenge as we lose ingredients to resistance, new regulatory requirements related to public acceptance, and due to commercial considerations. Furthermore, the problem of losses due to insect pests does not end in the field or with the harvest. Insects reduce the quality of stored grain and other stored products, and it is estimated that postharvest losses to corn and wheat alone amount to as much as $2.5 billion annually. Imported commodities as well as those destined for export must be protected from native and exotic pests. Exotic insect and weed pests that threaten our food, fiber, and natural ecosystems are another mounting concern due to the increase in world trade and travel. Invasive species directly threaten our agricultural crops, transmit devastating bacterial and viral diseases that threaten entire agricultural industries, and decimate our forests and urban landscapes; while invasive weeds reduce biodiversity, displace native species, and cost billions of dollars to control.

The goals of NP 304 are twofold: to understand the biology, ecology, and impact of these pests on agricultural production and natural systems and to develop, improve, and integrate environmentally safe technologies to exclude, eradicate, or manage pest populations. Priority is placed on sustainable and integrated practices that enhance the productivity, quality, and safety of U.S. agriculture while protecting natural resources, native ecosystems, human health, and the environment.

This National Program is divided into four research components:

- **Component 1: Systematics and Identification**: accurately identifying insects, mites, and weeds, whether native or invasive, to get important information about their possible
country of origin and bionomics, and the taxonomy and systematics of microorganisms associated with these insects and weeds, for aid in developing microbials as biological control agents

- **Component 2: Weeds**: improving existing and/or developing new, innovative control strategies for pests in traditional and organic agricultural and horticultural systems

- **Component 3: Insects and Mites**: preventing, managing, and controlling critical insect pests and weeds that threaten environmental areas and the agricultural areas bordering them

- **Component 4: Protection of Postharvest Commodities, Quarantine, and Methyl Bromide Alternatives**: contributing to the development of effective and sound management strategies to reduce pest damage that occurs after harvest, to limit the spread of exotic pests on agricultural commodities, and to ensure U.S. competitiveness in the international commerce of agricultural commodities

Below are research accomplishments for this national program from fiscal year 2020. The results are presented under the components and problem statements of this program’s 2020-2025 Action Plan. The report below is not intended to be a progress report describing all research conducted during the 2020 fiscal year; rather it is an overview that highlights major accomplishments, some of which are based on multiple years of research (not all research projects will reach an “accomplishment” endpoint each year).

ARS welcomes your input regarding our ongoing research programs. If you have any questions, please do not hesitate to contact the National Program 304 team: Kevin Hackett (Kevin.Hackett@usda.gov), Robert Miller (robert.miller2@usda.gov), Joe Munyaneza (Joe.Munyaneza@usda.gov), Tim Rinehart (Tim.Rinehart@usda.gov), Roy Scott (Roy.Scott@usda.gov), Timothy Widmer (Tim.Widmer@usda.gov), or Stephen Young (steve.young@usda.gov).

**Component 1 – Systematics and Identification**

*Systematics research safeguards U.S. agriculture.* By leveraging international collaborations and cutting-edge methodologies, including artificial intelligence, phylogenomics, remote sensing data, and DNA barcoding, ARS researchers in Beltsville, MD, and Washington, D.C., provide the basic systematic infrastructure to protect U.S. agricultural fields and natural areas from invasion by exotic pests. This work entails creating large photographic sets and databases of tens of thousands of species of wasps, mites, bees weevils, fruit flies, other potentially invasive pests, and the plants that host them into systematic collections. These collections in turn make it easier for APHIS inspectors at U.S. ports of entry to quickly identify and intervene to prevent potential agricultural pests from entering the Nation. (NP304, C1, PS1a, Project Nos. 8042-22000-269, -271, -289, -290, -292, -294-00D)
User-friendly economic assessment of rangeland prevention programs. Understanding the cost-benefit ratio of prevention programs is essential to the design of effective invasive weed management programs. ARS researchers in Burns, OR, developed an easy-to-use economic model to assess potential savings in livestock forage that might result from implementing prevention programs. The model can be used to determine potential loss in forage production caused by invasive plants and to estimate potential income savings by preventing invasive plant infestations. The model shows that savings per animal unit month (AUM) increases as the size of the initial infestation decreases, suggesting that prevention should focus on eliminating seed sources and seed production. Now for the first time, land managers will be able to assess the cost effectiveness of their invasive plant prevention programs on an individual basis. (NP304, C1, PS1a, Project No. 2070-22000-005-00D)

Genes found for determining the number of European corn borer (ECB) generations. Most U.S. farmers control ECB by planting corn varieties encoded to produce an insecticidal protein from *Bacillus thuringiensis* (Bt). Populations of univoltine (one generation per year) and multivoltine (two or more generations per year) ECB occur together in many parts of the United States and are reproductively compatible, but adults of these voltinism types often do not mate freely with each other because they are short-lived and are not always present at the same time during the season. Development of resistance to Bt corn by ECB is a great concern, and levels of gene exchange between locally adapted voltinism types can affect how fast and how far Bt resistance spreads once it develops in a population of one of them. ARS researchers in Ames, IA, in collaboration with Tufts University and University Massachusetts-Dartmouth colleagues (funded in part by National Science Foundation) have discovered the genes responsible for controlling the number of generations, the first time this has been accomplished for any species of moth. The results have opened the door to developing genetic markers to identify the voltinism type of any field-collected ECB, something that cannot be accomplished visually because the types appear identical. This capability will be crucial in estimating rates of gene flow between the voltinism types in different areas, and thus the rate of spread of adaptations like Bt resistance across a landscape or region. (NP304, C1, PS1a, Project No. 5030-22000-018-00D)

Molecular profiling of citrus leaves: a new tool for early detection of citrus greening disease. Citrus greening is devastating orchards in Florida and the south, and threatens groves in California. A collaboration between ARS scientists in Ithaca, NY, and Riverside, CA, identified molecular changes in citrus leaves resulting from infection with the causative citrus greening bacterium. Leaf samples were collected biweekly from lemon and navel orange trees for a year following graft inoculation with either pathogen-containing or healthy budwood and analyzed for biomarkers of infection. RNA, protein, and metabolite levels were compared over time between healthy and infected plants, revealing molecular profiles that were associated with infection months before visual symptoms of disease appeared. Results from this study reveal differences in the response to infection between these two distinct varieties of citrus and provide insight into how the plant response to the pathogen changes with time. These findings can be used to improve diagnostic tests for citrus greening disease. This high-profile research
was featured on the cover of a 2020 issue of *Journal of Proteome Research*. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

**Pheromone system, not ecology, is responsible for maintaining strain differences in European corn borer (ECB).** ECB is a major pest of corn, which most U.S. farmers control by planting corn varieties that produce an insecticidal protein from a gene introduced from the bacterium *Bacillus thuringiensis* (Bt). There are two strains of ECB, which use either a “Z” or “E” pheromone communication system. Although mating between individuals of different strains is possible in regions where both the Z and E strains co-occur, hybridization is partly curtailed by preference of males for females of their own pheromone type. However, the strains differ ecologically in other ways that might also discourage inter-strain mating, such as host plant preferences and possibly adult dispersal behavior. ARS researchers in Ames, IA, in collaboration with the University Massachusetts-Dartmouth, Tufts University, Iowa State University, Jilin Academy of Agricultural Sciences, and Pennsylvania State University (partly funded by NSF and USDA National Research Initiative) examined variation in the genomes of both Z and E pheromone strains and concluded that only the genomic regions containing the genes responsible for pheromone production by females and pheromone response by males are involved in maintaining differences between the strains; ecological factors are not responsible. This information is important for modeling ECB population dynamics and gene flow between pheromone strains in different areas, and thus the rate of spread of adaptations like Bt resistance across a landscape or region. (NP304, C1, PS1a, Project No. 5030-22000-018-00D)

**Use of fungal pathogens to control whitefly population on cotton.** The whitefly is an important insect pest of many crop families, including cotton, cucumber, and cabbage. ARS scientists in Ithaca, NY, in collaboration with ARS colleagues in Byron, GA, and the University of Georgia, identified a fungus that kills whiteflies on cotton as *Isaria javanica*. This newly discovered fungus caused greater mortality than the commercial fungus used against whiteflies. It shows promise in biocontrol of whiteflies and other difficult-to-control sap-sucking insect pests of agriculture crops. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

**Understanding “catapult” motion mechanism of pest flea beetles.** Flea beetles are plant feeders that belong to a highly diverse group of about 9,900 species, many of which are serious pests and feed on crops and destroy valuable plants and cost millions of dollars annually. Most flea beetles live, feed, and procreate on the upper leaf surface of their host plants, thus making them vulnerable to predators, including birds, ants, and spiders. An ARS researcher in Washington, DC, working in collaboration with entomologists, physicists, and engineers from the University of Florida and various institutions in China described the apparatus hidden inside the beetle’s hind leg that helps them to escape predation. The apparatus is a highly efficient catapult that can propel the beetles to a distance hundreds of times their body length, with an acceleration that can reach 865 times the acceleration of gravity. This mechanism is among the key traits responsible for the flea beetle’s remarkable species diversity and overall high evolutionary success that includes the ability to feed on a wide range of plants. The catapulting jump mechanism is so efficient, and yet simple, that it might find an excellent use in robotics, as
Identification of peptides for control of the insect vector of citrus greening. ARS scientists in Ithaca, NY, published a comprehensive description of the peptidome of the vector of HLB (Huanglongbing, aka citrus greening), the Asian citrus psyllid (*Diaphorina citri*), which included hundreds of molecules with classic signatures of biologically active peptides. This data collection is a rich resource for the development of novel antimicrobials, and for more than 100 candidate insect neuropeptides. This work led to an APHIS-funded HLB initiative to screen a series of these neuropeptides, some of which have demonstrated insecticidal activity against other hemipteran insects, as new and environmentally friendly pesticides for the management of the psyllid in citrus. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

**Component 2 – Weeds**

Establishment of a new biological control agent of Cape-ivy. Cape-ivy (*Delairea odorata* Lem.) is a vine-like perennial weed that has invaded sensitive coastal streambank, forest, and scrub habitats along the California coast, and it is also invasive in Hawaii, Australia, and southern Europe. Cape-ivy smothers native herbs, shrubs, and trees and can clog coastal streams that supply agricultural water. ARS scientists in Albany, CA, released a fly from Cape-ivy’s native range in South Africa that makes tumor-like galls in Cape-ivy’s shoot tips, which reduces shoot tip abundance and growth. The fly is the world’s first biological control agent against Cape-ivy. Releases were conducted at 18 field sites in California between late 2016 and 2019. The fly has established large populations at four sites, including two California State parks, with gall abundance increasing 40-fold since early 2019. Continued dispersal and galling will decrease Cape-ivy’s competitive advantage and invasiveness. (NP304, C2, PS2b, Project No. 2030-22000-027-00D)

Community-based herbicide-resistant weed management. Evolution of herbicide resistance in weeds is one of the top challenges facing U.S. farmers. ARS scientists in Urbana, IL, and Beltsville, MD, developed a spatial-temporal model describing the impacts of 1) farm-scale weed management strategies on rates of herbicide resistance evolution, and 2) coordinated management practices on the spread and occurrence of herbicide resistance. These findings demonstrate the importance of large-scale cooperative management strategies that diversify herbicide mode of action and frequency of glyphosate use. This work informs researchers, industry, and growers on the importance of community-based weed management. (NP304, C2, PS2a, Project No. 8042-22000-166-00D)

New assessment technology improves evaluation of aquatic weed management success. ARS scientists in Davis, CA, have found a way to evaluate methods used to control submersed aquatic weeds such as Brazilian waterweed. By combining data from various sources, the scientists were able to conduct a rigorous test of the effectiveness of weed management
practices and related changes in species composition of native plant communities. This new technology uses scanning and SONAR data to generate maps that help predict the need for weed control and quantify the effectiveness of actions taken to reduce weed impacts. Output from these tools also provides an effective way to communicate results to regulatory agencies, and to stakeholders and the public. California State Parks is now using the technology. (NP304, C2, PS2b, Project No. 2030-22000-029-00D)

**Release of new biological control agent of yellow starthistle.** Yellow starthistle is a spiny toxic weed that has invaded more than 19 million acres of rangeland in the western United States, costing an estimated $1.4 billion in California alone. It reduces forage for livestock, decreases desirable vegetation, and increases the risk of wildfire. Although six species of insects have been introduced that attack the flower heads to reduce seed production, we still need an agent that attacks roots and the immature rosettes (leaves). In rigorous laboratory tests in the United States, and field tests in the plant’s native Mediterranean range, ARS scientists in Albany, CA, with collaborators, demonstrated that a rosette weevil poses no significant risk to nontarget native wild plants or to native or nonnative crops. Consequently, APHIS issued a release permit, and the first release of the weevil as a biological control in the world was made in California in April 2020. Widespread establishment of this weevil should reduce yellow starthistle densities. (NP304, C2, PS2b, Project No. 2030-22000-027-00D)

**Detection of microbial genes for reduction of greenhouse gas in soil.** Contrary to the decades-long knowledge that denitrifying bacteria were the only agents responsible for the biological reduction of dinitrogen oxide (N₂O) to gas (N₂), ARS scientists in Urbana, IL, in collaboration with University of Illinois Urbana-Champaign partners discovered that this microbial activity can also be mediated by highly diverse bacteria, previously unaccounted for in natural soil, sediment, and water. They determined that the functional gene \( \text{nosZ} \) was effective at denitrification and designed and validated molecular probes to detect these microbial genes in the soil. These molecular probes were designed to be compatible with new high-throughput microfluidic array gene sequencing technologies, enabling large numbers of samples to be analyzed with multiple different gene targets. This advancement now allows researchers to conduct comprehensive studies of the biological fate of nitrogen fertilizers in agricultural systems and soil N₂O emissions. (NP304, C2, PS2a, Project No. 5012-12220-009-00D)

**Biological control of the cactus mealybug pest in Puerto Rico.** The Harrisia cactus mealybug (HCM) was introduced into Puerto Rico around 2000 and is destroying several native cactus species in dry forest habitat there. HCM has also invaded Florida (feeding on amaranth plants) and California (feeding on cactus), causing concerns for the safety of native cactus in North America. To identify the source of the cactus-feeding mealybugs, ARS researchers in Gainesville, FL, collected HCM from various locations around the world and characterized them using genetic identification. The original HCM species group was found to consist of five different species with each species feeding on specific host plants. The mealybugs feeding on species of amaranth plants in Puerto Rico and Florida originated from northeastern Brazil and were a nonpest invasive species. The mealybugs infesting cactus in Puerto Rico were an invasive species from southeastern Brazil and genetically identical to cactus-feeding mealybugs found in
California. Fortunately, the California pest mealybug population was eradicated by State authorities, but a concern exists that additional introductions will occur. Knowing the geographic origin of the Puerto Rican mealybug focuses the need to search for natural enemies to serve as biological control agents from southeastern Brazil before another invasive introduction occurs in North America. (NP304, C2, PS2b, Project No. 2015-6036-22000-031-00D)

**Long-term ecological effects of a released biocontrol agent were assessed.** As part of ARS’s commitment to long-term research, particularly the establishment of biocontrol agents, ARS researchers from Reno, NV, monitored saltcedar ecosystem responses to the introduction of the northern tamarisk leaf beetle, a biological control agent of saltcedar. This has produced 12 years of data on small mammal diversity, stand-level carbon dioxide exchange and evapotranspiration rates, and understory plant community response. Ecosystem responses of carbon and water cycling showed a pattern of plant recovery in the last 3 years relative to the previous 9 years. (NP304, C2, PS2b, Project No. 2060-22000-024-00D)

**Post-wildfire rehabilitation strategies assessed.** On average, $35 million per year is spent on post-fire rehabilitation treatments by the Bureau of Land Management (BLM) to reduce annual grass invasion and re-establish native communities that are resilient to future wildfire. Using a combination of field work and spatial analysis, ARS scientists in Reno, NV, examined the effects of fire, rehabilitation, and environmental variables on plant communities, cheatgrass invasion, and changes in fire regimes. Both elevation and aridity influenced cheatgrass invasion and future wildfire. Aerial seeding tended to increase future wildfires compared to drill seeding and planting of diverse bunchgrass communities after wildfire and rehabilitation were most successful in reducing cheatgrass invasion. These results suggest that post-fire rehabilitation treatments should focus on using a diverse assemblage of native grasses to reduce invasion by exotic annual grass species. These techniques are being used by BLM on more than 70,000 acres at the 2018 Martin Fire site with demonstrated success in establishing desired species and reducing cheatgrass density. (NP304, C2, PS2b, Project No. 2060-22000-024-00D)

**Glyphosate does not affect soil microorganisms.** The herbicide glyphosate has become a nearly ubiquitous component of agricultural production across the globe, enabling an increasing adoption of no-till agriculture. Despite this widespread use, there remains considerable debate on the consequences of glyphosate exposure. In collaboration with university partners, ARS researchers in Beltsville, MD; Stoneville, MS; and Urbana, IL, compared the microbial communities associated with crop roots in a variety of organic and conventional corn and soybean farming systems. No effects of glyphosate were found on soil microbial communities associated with glyphosate-resistant crop varieties. This research was replicated in both time and space in realistic farming systems with well-documented glyphosate use histories. The findings provide research-based knowledge that the practice of no-till agriculture, enabled by use of the glyphosate herbicide, does not pose undue risk of negatively altering soil microbial communities. (NP304, C2, PS2a, Project No. 5012-12220-009-00D)

**Biological control agent approved for release against invasive melaleuca tree.** Melaleuca is an invasive tree that is threatening the Florida Everglades. Due to environmental concerns and the
widespread invasion, herbicides are not an option for control. Biological control with insects has been very successful using agents developed by the ARS Australian Biological Control Laboratory. A new gall midge was discovered that damages melaleuca and complements the existing biological agents currently in use. After testing in Australia and quarantine at ARS facilities in Fort Lauderdale, FL, a petition to release this insect was prepared and approved by APHIS for the biological control of weeds. Release of this insect will give land managers another option to further manage this invasive tree. (NP304, C2, PS2b, Project No. 0206-22000-003-00D)

Diet-toxicity bioassays of western and northern corn rootworm developed. Western and northern corn rootworms are rapidly evolving resistance to all commercially available genetically modified corn varieties. The U.S. Environmental Protection Agency has mandated that registrants of *Bacillus thuringiensis* (Bt) crops develop resistant colonies to facilitate understanding mechanisms that pests may use in surviving Bt crops. Currently, diet-toxicity evaluations of each major maize seed company’s Bt toxins are conducted on proprietary artificial diets, which prevents direct comparisons of toxicity data from different seed companies. ARS researchers in Columbia, MO, developed advanced high-throughput design and screening methods to formulate high-performing diets for use in diet-toxicity bioassays of the rootworms. Several industry and academic researchers were trained on site to prepare and use the diets that had been developed by ARS researchers. A Phase I Small Business Innovation Research grant was awarded to the collaborative group to further improve the packaging and shipping stability of both diets. These diets benefit researchers and industry and extension workers who must rapidly, inexpensively, easily, and reproducibly assess rootworm resistance against genetically modified corn in the field, and evaluate new toxins using diet-toxicity assays. (NP304, C3, PS3c, Project No. 5070-22000-037-00D)

**Component 3 – Insects and Mites**

Plant “organ transplants” offer a new way of delivering genetic engineering solutions to solve crop pest/pathogen problems. There is an urgent need for solutions to control whitefly and the diseases transmitted by it. ARS researchers in Fort Pierce, FL, in collaboration with a private industry partner, developed a method of engineering only a group of plant cells that can be attached to other plants (essentially as a new organ) to produce desired molecules that are secreted into the plant vascular tissue and move throughout the plant. This “new organ” cannot survive away from the plant and does not move from the location where it is attached, thus the harvested commodity (i.e., fruit, nut, etc.) is not genetically engineered. It also cannot form whole plants, seed, or pollen, thus there is no escape of genetic material. The scientists are evaluating the ability of this strategy to cure trees infected with Huanglongbing (HLB, aka citrus greening) by engineering similar organs to produce natural peptides and double-stranded RNA that kills the HLB-causing bacterium, and attaching these organs to ornamental and/or horticultural crops. Proof-of-concept has been completed in tomato. This strategy could be adapted as a means to rapidly deliver genetic engineering solutions in an environmentally sustainable and consumer acceptable method. (NP304, C3, PS3c, Project No. 6034-22320-003-00D)
Beneficial nematodes complete their first trip to space. In an exciting collaboration between ARS researchers in Byron, GA, and industry partners, beneficial insect-killing nematodes (small roundworms) were sent to the International Space Station in support of the goal of developing environmentally friendly methods to support long-term space travel. Beneficial nematodes, also called entomopathogenic nematodes, are alternatives to using broad-spectrum chemical insecticides and are also safe to humans and other nontarget organisms. They are used to control a wide variety of insect pests on Earth. The nematodes sent into space were capable of navigating through soil and killing insect pests. This was the first biological control experiment in space. The mission represents a look into the future where food crops will be grown in space. (NP304, C3, PS 3a, Project No. 6042-22000-023-00D)

Integrated West Nile virus (WNV) early warning surveillance system developed. West Nile fever/encephalitis is the most important mosquito-borne disease in the continental United States. The disease is caused by a flavivirus that is separated into distinct lineages, with lineage 1 (L1) and lineage 2 (L2) encompassing all WNV known isolates associated with human and veterinary disease. Currently, all known U.S. WNV isolates belong to L1. L2 isolates, usually found in sub-Saharan Africa, were recently found in Europe and caused large human and equine WNV outbreaks. The invasive threat and risk of WNV L2 invading the United States is significant because recent evidence has demonstrated that North American mosquito species are competent vectors of WNV L2 isolates from Africa and Europe. ARS scientists in Greece associated with the European Biological Control Laboratory designed an integrated WNV early warning surveillance system specifically targeting the L2 strains. The system relies on detecting viral RNA in field-collected mosquitoes and screening sentinel chickens for WNV-specific antibodies. The surveillance system was successfully implemented and provided information on WNV mosquito circulation and enzootic transmission 1 month prior to human cases, thereby allowing for targeted and proactive vector control interventions. Knowledge of the WNV L2 ecology in Europe combined with optimized field-based surveillance systems and laboratory diagnostic tools can be applied to enhance early detection and early warning systems to control and reduce this emerging threat. (NP304, C3, PS3a and PS3b, Project No. 0212-22000-027-00D)

Hormone mimics provide effective safe, selective aphid control. Aphids are significant agricultural pests in temperate regions, causing yellowing, mottled leaves, stunted growth, curled leaves, low yields, and death through disease transmission. New approaches are needed for effective aphid management and control. ARS researchers in College Station, TX, in collaboration with researchers from Belgium and the United Kingdom, developed stable mimics of two neuropeptide hormones that when applied together as a spray in the field cause large-scale mortality in the peach potato aphid. Notably, this treatment was shown to be selective, and did not harm beneficial insects such as natural enemies and pollinators. These neuropeptide hormone mimics can be recommended as environmentally safe aphid management agents. (NP304, C3, PS3a and 3b, Project No. 3091-22000-033-00D)

Agricultural byproducts improve cricket and mealworm production for animal feed. Massive quantities of agricultural byproducts are produced annually in the United States. Most of these products, such as distilled grains for ethanol production, rice bran, and extracted grain meals
from vegetable oil production, are not fit for human consumption. However, some insects can feed on these products and convert them to high-quality protein that could be an important alternative to fish meal in the formulation of feeds for farm animals and aquaculture. Insect production, however, is expensive. Using agricultural byproducts to produce insect diets could reduce the cost of insect production. ARS researchers in Stoneville, MS, developed diets for the house cricket and the yellow mealworm using 80 to 90 percent agricultural byproducts. Researchers there further developed means for producing larger mealworms by selecting (essentially domesticating) larger stock insects over 8 years. Together, these insects and byproduct-based diets resulted in insects of better quality, and potentially competitive with fish meal, an unsustainable source of animal protein currently in use. These new diets will benefit the fast-growing insect production industry. Other industries, such as ethanol, vegetable oil, etc., will also benefit via the use of their byproducts, which could become a commodity. (NP304, C3, PS3a, Project No. 6066-22000-086-00D)

**Friendly fungus provides protection to pecan trees.** Certain beneficial insect-killing fungi are used as natural bioinsecticides. These fungi are produced commercially and can kill a wide variety of important insect pests including pecan weevil and pecan aphids. However, the fungus can be costly to apply. ARS researchers in Byron, GA, discovered that the fungus can be introduced into the pecan tree as an endophyte that can live inside the tree. As an endophyte, the fungus provides built-in protection to the tree. Initial studies indicate the endophytic pecan trees have potential to suppress aphid populations and other pests. This technology could be used as an efficient and environmentally friendly approach to control pecan pests. (NP304, C3, PS3a, Project No. 6042-22000-023-00D)

**Genetic breakdown of a conditional lethality system for insect population control.** Use of genetically modified insects presents an attractive alternative to conventional pest control methods that employ the sterile insect technique. One concern in the use of genetically modified insect strains is that we do not know whether spontaneous mutations or resistance to the genetic modification may occur when many millions of insects are released for pest management purposes. Researchers in Gainesville, FL, examined the common fruit fly, Drosophila melanogaster, and found that when extremely large numbers of insects are produced, some survivors developed spontaneous mutations and inherent resistance to the genetic manipulation for lethality. From these results, the researchers estimated that from 100 million genetically modified fertilized eggs deposited in the field, several hundred adults may survive, and that these resistant survivors and their descendants may be expected to replace the susceptible field populations, which will require alternative methods for control. To avoid the development of populations that would be resistant to a single conditional lethality system, a secondary redundant lethality system is needed that would prevent insects from becoming resistant to either one of the systems to survive. (NP304, C3, PS3b, Project No. 6036-22000-030-00D)

**Susceptibility of northern corn rootworm to Bt corn toxins established.** The northern corn rootworm (NCR) is an economic pest in the Midwest and is currently managed primarily by growing transgenic corn that expresses insecticidal proteins from a *Bacillus thuringiensis* (Bt)
bacterium. The U.S. Environmental Protection Agency requires registrants of rootworm-targeted Bt products to develop and implement a resistance management plan for NCR. To help meet this requirement, ARS researchers in Brookings, SD; Columbia, MO; and University of Missouri conducted efficacy tests against NCR using Bt plants and with artificial diets for the rootworm overlaid with one of four different Bt toxins. These tests showed that NCR was susceptible to each of the four Bt toxins. The findings are important to companies that produce corn hybrids and to researchers for establishing baseline levels of toxicity against NCR and for monitoring resistance evolution in field populations of the rootworm. (NP304, C3, PS3d, Project No. 3080-21220-006-00D)

Managing zebra chip using field-collected specimens of potato psyllid vector to identify natural plant reservoirs of the disease pathogen. Zebra chip, an economically important disease of potato vectored by potato psyllids, is difficult to manage. ARS researchers in Wapato, WA, in collaboration with scientists at Texas A&M University, developed new molecular tools that allow them to identify plant remains in field-collected psyllids while also simultaneously confirming the presence or absence of the zebra chip pathogen in those same specimens. Significant correlation between the presence of a specific plant species and the pathogen in psyllid populations is evidence that psyllids feed on those plants and acquire the zebra chip pathogen from those plants. Those data are being used to guide field efforts to eradicate weeds in potato growing regions that harbor the zebra chip pathogen. This new pest management tool is a great benefit to the $4 billion U.S. potato industry. (NP304, C3, PS3a, Project No. 2092-22000-021-00D)

Enhanced artificial diets for the pink spotted lady beetle. The pink spotted lady beetle serves as a good biocontrol agent because it feeds primarily on aphids. In the past, artificial diets for insect predator breeders have been ineffective in producing quality predators due to nutrient deficiencies in the diets. ARS researchers in Stoneville, MS, developed new artificial diets for the pink spotted lady beetle using insect components derived from commercially available insect powders. Powders produced from yellow mealworm, house cricket, and house fly were incorporated into experimental artificial diet formulations and tested for their efficacy at producing quality lady beetles. These new diet formulations can be produced in high quantity at a reasonable price and constitute a promising solution to the costly techniques currently used to produce lady beetles and other beneficial insect predators. This diet formulation could reduce the price of biological control agents and reduce the need for insecticide applications, which would benefit the environment and make agriculture more sustainable. (NP304, C3, PS3a, Project No. 2015 6066-22000-086-00D)

Biological control of arthropod pests has large economic value. Biological control is an integral tactic of modern integrated pest management, but we have only a rudimentary knowledge of its economic value. Collaborative research between ARS scientists in Maricopa, AZ, and University of Arizona colleagues synthesized available economic data on projects targeting arthropod pests and found that introductory biological control of exotic pests is valued at $37.3M per project with an average benefit to cost ratio of 61:1. The augmentation biological control industry is valued at $1.7 billion and is growing at 15 percent annually, while the value
of conservation biological control is around $74 per hectare. Interaction among diverse scientists and stakeholders will be required to ultimately measure the inclusive benefits and costs of biological control. However, focus on gaining greater accuracy in measurement should be balanced with additional efforts to educate both end users and public institutions about the immense value of biological control in order to spur its greater adoption. (NP304, C3, PS3b, Project No. 2020-22620-022-00D)

**Collection data extends known geographic range of eight lady beetle species.** Most lady beetles are voracious predators of insects, mites, and aphids. Despite their importance as natural enemies, fundamental knowledge of many lady beetle species is lacking, including up-to-date information about their geographic distributions. In two studies, ARS researchers in Brookings, SD, with colleagues at Chadron State University in Nebraska, scrutinized lady beetle insect collections at various institutions and discovered new records that extended the known geographic distribution of eight species of predatory lady beetles in the United States. These findings confirm the importance of insect collections as fundamental and valuable resources on lady beetles and support the notion that systematic updated analysis of collection data will yield new insights into their geographic ranges and improve our understanding of the beetle's diversity. (NP304, C3, PS3d, Project No. 3080-21220-006-00D)

**Discovery of diagnostic genetic markers for cryptic invasive agricultural fly pests.** Apple maggot is a serious pest of apples that affects U.S. exports, especially from the Pacific Northwest. This insect pest is commonly monitored using traps. The similar-looking snowberry maggot fly, a non-apple pest, is frequently caught on these traps and can be mistaken for apple fly, which could lead to unjustified quarantines. ARS researchers in Wapato, WA, in collaboration with researchers at the University of Notre Dame in Indiana, Wayne State University in Detroit, MI, and McGill University in Montreal, Canada, developed inexpensive, quick, and reliable methods for discriminating the two fly species. A simple and cost-effective diagnostic approach using DNA marker and polymerase chain reaction was able to distinguish the fly species. These new tools provide an important and effective strategy for correctly identifying apple maggot and other cryptic pests, and a great benefit to national and international trade of apples and other fruits. (NP304, C3, PS3a, Project No. 2092-22430-002-00D)

**Density management techniques for mealworm production.** Mealworms are used in animal feed and as an ingredient in some human foods, but current mass production techniques make insect protein expensive. ARS researchers in Stoneville, MS, have developed a new rearing method of mass producing mealworm *Tenebrio molitor* using density management techniques that may result in substantial cost reductions and a subsequent price reduction of mealworms. (NP304, C3, PS3a, Project No. 6066-22000-086-00D)

**Sublethal insecticide effects on Bt soybean refuge strategy.** High Bt (*Bacillus thuringiensis*) protein dose and deployment of a non-Bt crop refuge are key strategies for delaying resistance in target pests to Bt crops. However, insecticide sprays are often needed in the non-Bt refuge to manage the target pest. A collaborative study between ARS researchers in Maricopa, AZ, and the University of Sao Paulo, Brazil showed that sublethal doses of two common insecticides
used in Brazilian soybean systems negatively affected population growth and flight behavior of a key soybean pest. These biological effects could reduce the abundance and movement of susceptible moths from refuge fields. Results are of value to growers and regulators interested in sustaining the durability of this pest control technology. (NP304, C3, PS3b, Project No. 2020-22620-022-00D)

Coffee berry borer management app developed in Hawaii. The coffee berry borer (CBB) insect is the most economically important coffee pest worldwide, including Hawaii. The life cycle of CBB inside the host plant makes these insects difficult to monitor and control. Using 3 years of monitoring data from 21 sites on Hawaii island plus more than a dozen more on Oahu and Puerto Rico, ARS researchers in Hilo, HI, developed a data collection set and implemented a mobile application that allows growers to monitor their fields for CBB and offers guidance on its management. A technology transfer agreement is currently being developed with a private company that is producing weather sensor networks in Hawaii to allow further development and public availability of the app. (NP304, C3, PS3c, Project No. 2040-22430-026-00D)

Gene editing used to tag toxin transporter genes in the Indian meal moth and fall armyworm. Resistance to toxin-based insecticides have become a major issue for biologically engineered crops. ARS researchers in Gainesville, FL, used gene editing to introduce a fluorescent gene marker into the Indian meal moth and the fall armyworm to create mutations in genes that are important in acquisition of toxin resistance. Gene editing of these transporter genes resulted in cultured insect cell lines and white eyed moths that produced a fluorescent protein marker and affected Bt resistance. Using this gene editing system, various pesticide resistance genes can be mutated, and the effects studied to find mechanisms to avoid or reverse pesticide resistance. (NP304, C3, PS3b, Project No. 6036-22000-030-00D)

Refuge requirements for a new transgenic cotton line determined. A new Bacillus thuringiensis (Bt) toxin was specifically developed against the tarnished plant bug, and the toxin also has activity against several other insect pests, including the cotton fleahopper. But before transgenic cotton expressing this new toxin can be released commercially, a management strategy to delay or prevent resistance to the toxin must be approved by the U.S. Environmental Protection Agency for all targeted insects. ARS researchers in College Station, TX, in collaboration with researchers at Texas A&M University, compared the genomic structure of cotton fleahopper populations in cotton and those in nearby patches of wild weed hosts throughout the year. Based on the abundant numbers of cotton fleahoppers found in nearby weed hosts, and the high level of intermixing among cotton fleahopper populations in weed hosts and cotton fields throughout the growing season, the studies indicated that it is not necessary to plant non-Bt cotton plants to serve as a refuge in the proximity of the Bt cotton field. Instead, nearby wild weed hosts that harbor plant bugs could serve as a natural refuge for the pests in the new Bt cotton system. This work provides a way to assure proper regulation of the Bt technology while avoiding needless and costly restrictions. (NP304, C3, PS3a and 3b, Project No. 3091-22000-033-00D)
Use of the artificial sweetener erythritol to manage pear psylla. Pear psylla is a pest that requires repeated applications of insecticides to control it. New management tools are needed to reduce the risk of pear psylla populations developing resistance to current insecticides. The artificial sweetener erythritol is nontoxic to humans but is insecticidal when ingested by certain insects. ARS researchers in Wapato, WA, with colleagues at Heritage University in Toppenish, WA, examined whether erythritol is also lethal to pear psylla. Results of laboratory and field experiments showed that treatment of pear with 20 percent erythritol causes 60–80 percent mortality of pear psylla nymphs and adults. These findings demonstrate that erythritol could be developed into a safe and effective tool for the management of pear psylla and is a great benefit to the tree fruit industry. (NP304, C3, PS 3a, Project No. 2092-22430-002-00D)

Food resources enhance performance of wheat stem sawfly parasitoids. Despite almost a century of research, the wheat stem sawfly continues to be a major pest of wheat. Biological control with tiny parasitoid wasps has promise, but the adult wasps need food, especially sugar. ARS researchers in Sidney, MT, found that incorporating buckwheat into cover crop mixes could benefit parasitoid performance in adjacent wheat crops. Studies continue on providing aphid honeydew in habitat management strategies to improve parasitoid conservation for sawfly control. (NP305, C3, PS3b and PS3d, Project No. 3032-22000-018-00D)

Pink bollworm resistance to Bt cotton involves multiple genetic and biochemical mechanisms. Crops that express a toxic Bacillus thuringiensis (Bt) protein are critical components in the control of important insect pests, but evolving resistance threatens their continued use. Pink bollworm is a global pest of cotton and multiple strains have developed resistance to Bt toxins. ARS scientists in Maricopa, AZ, with researchers from the University of Arizona, show that the primary mechanism of pink bollworm resistance is mutations in the receptors that enable Bt toxin to bind to the insect midgut. These mutations involve changes to the genetic sequence and how the genes are translated into proteins. Reduced receptor production and improper localization of those proteins on cell surfaces also can impair Bt toxin efficacy. These findings show the notable adaptability of pink bollworm to evolve resistance to Bt cotton and demonstrate the challenges for monitoring and managing resistance to Bt crops. The results are valuable to scientists concerned with understanding the mechanisms of resistance, to private industry for developing new commercial strategies to target pests, and to government authorities with responsibility for regulating transgenic crops. (NP304, C3, PS3b, Project No. 2020-22620-022-00D)

Parents time egg development in Mormon crickets. Most insects lay eggs that hatch the following year. ARS scientists in Sidney, MT, were the first to determine that Mormon crickets in the Bighorn Mountains of Wyoming, where they compete with cattle for summer pasture, laid eggs with delayed embryonic development and hatching when the parents were exposed to a short-day photoperiod, indicative of the end of the growing season. By delaying the development of their eggs in the soil, females create an egg bank, or a kind of refuge for their offspring to survive the harsh conditions of winter and persist at high altitudes where the growing season is short. State entomologists report that knowledge of egg bank longevity
improves forecasting and reduces the cost and impact of management of this pest. (NP304, C3, PS3b and PS3d, Project No. 3032-22000-018-00D)

**Varroa mite found to be sensitive to carbon dioxide.** Varroa mites feed and live on adult honey bees and feed and reproduce on larvae and pupae, transmit numerous viruses, and contribute to a yearly loss of more than 40 percent of U.S. managed honey bee colonies. ARS researchers in Gainesville, FL, in collaboration with researchers at the University of Florida have discovered a vulnerability in the mite that might be exploited. The researchers found that the mite is sensitive to carbon dioxide. When mite-infested honey bees were exposed to low-dose carbon dioxide the mites became anesthetized and dropped from the bees. This technique will be evaluated for its application to the entire hive. Alternative control measures are needed to replace current pesticide use due to resistance and honey bee exposure. (NP304, C3, PS3c, Project No. 6036-22000-028-00D)

**Discovery of a chemical repellent that targets the coffee berry borer.** The coffee berry borer (CBB) has a cryptic life habit inside coffee berries. ARS scientists in Beltsville, MD, identified a compound that acts as a repellent against CBB. In a field experiment in Hawaii, the repellent resulted in up to an 80 percent decrease in borer captures in traps with an attractant compared with traps containing just the attractant. The repellent was placed inside a plastic wrapper that allowed the repellent to exit at controlled rates and to remain active for at least 19 weeks. These findings have potential national and international impact for coffee producers, the coffee industry, and consumers. (NP304, C3, PS3a, Project No. 8042-22000-287-00D)

**RNA interference (RNAi) is effective in lygus by injection but not by ingestion.** Getting agricultural pests such as lygus (tarnished plant bugs) to ingest double-stranded RNA (dsRNA) to trigger RNAi-based knockdown of target genes has been promoted as a novel control strategy. However, studies to explore how RNAi methods would work in lygus by ARS researchers in Maricopa, AZ, established that current methods for oral delivery of dsRNAs are ineffectual. Extraoral degradation of dsRNAs accounts for the poor efficacy, indicating that the barrier is related to the persistence and uptake of the ingested dsRNAs. These results will facilitate the development of highly targeted control approaches for lygus using gene manipulation, which would make it possible to reduce pesticide application for cotton and other commodity crops for which they are pests, and will be particularly of interest to those developing RNAi-based strategies that depend on oral delivery. (NP304, C3, PS3b, Project No. 2020-22620-022-00D)

**Alfalfa is an effective trap crop for strawberry fields.** The lygus bug is a major strawberry pest but it prefers to feed on alfalfa. As such, embedding strips of alfalfa within large strawberry production fields can serve as a sink for both lygus and its predaceous natural enemies. ARS researchers in Maricopa, AZ, examined the population dynamics and dispersal characteristics of the lygus predator complex in strawberry fields embedded with 1 row of alfalfa for every 49 rows of strawberry. They found that the minute pirate bug accounted for 84 percent of the predator population. Predator movement from a centralized alfalfa trap crop row showed that most predators dispersed less than 2 meters, indicating that the prey reservoir found in trap crops often produces a predator sink. This study suggests that alfalfa is a useful cultural (trap
cropping) and biological (refuge for natural enemies) control tactic for managing lygus in strawberries, which will lead to less insecticide use and greater production of organically grown strawberries. (NP304, C3, PS3b, Project No. 2020-22620-022-00D)

**Biocontrol for emerald ash borer to protect North American ash trees.** The emerald ash borer is a serious invasive forest pest that has destroyed natural and urban ash forests and threatens the existence of North American ash species. ARS researchers in Newark, DE, introduced a parasitic wasp, *Spathius galinae*, which has established a self-sustaining population 3 years after its release. The wasp has now spread to other areas and is providing significant biocontrol of the borer in large ash trees in the northeast United States. This natural enemy of emerald ash borers is critical for the protection of large ash trees in the U.S. forest ecosystems. (NP304, C3, PS3d, Project No. 8010-22000-028-00D)

**Molecular profiling of citrus leaves: a new tool for early detection of citrus greening disease.** Citrus greening is devastating orchards in Florida and the south, and threatens groves in California. A collaboration between ARS scientists in Ithaca, NY, and Riverside, CA, identified molecular changes in citrus leaves resulting from infection with the causative citrus greening bacterium. Leaf samples were collected biweekly from lemon and navel orange trees for a year following graft inoculation with either pathogen-containing or healthy budwood and analyzed for biomarkers of infection. RNA, protein, and metabolite levels were compared over time between healthy and infected plants, revealing molecular profiles that were associated with infection months before visual symptoms of disease appeared. Results from this study reveal differences in the response to infection between these two distinct varieties of citrus and provide insight into how the plant response to the pathogen changes with time. These findings can be used to improve diagnostic tests for citrus greening disease. This high-profile research was featured on the cover of a 2020 issue of *Journal of Proteome Research*. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

**Development of a new trapping system for spotted wing drosophila (SWD) pest management.** ARS scientists in Beltsville, MD, collaborated with scientists at Towson University and in France, Germany, Spain, and Italy to develop a dry trap baited with a controlled-release attractant dispenser to attract SWD. The new trapping system demonstrated earlier SWD detection compared to the conventional liquid traps in use in orchards. Due to the simplicity of the dry trap design and its controlled release dispenser, the trap provides growers with an efficient, convenient, and easy processing tool to detect and control SWD infestation. (NP304, C3, PS3a, Project No. 8042-22000-288-00D)

**Use of fungal pathogens to control whitefly population on cotton.** The whitefly is an important insect pest of many crop families, including cotton, cucumber, and cabbage. ARS scientists in Ithaca, NY, in collaboration with ARS colleagues in Byron, GA, and the University of Georgia, identified a fungus that kills whiteflies on cotton as *Isaria javanica*. This newly discovered fungus caused greater mortality than the commercial fungus used against whiteflies. It shows promise in biocontrol of whiteflies and other difficult-to-control sap-sucking insect pests of
agriculture crops. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

A strategy to control Asian citrus psyllid. Growers rely on insecticides to control Asian citrus psyllid (ACP), but insecticide-resistant psyllid populations are emerging, and control costs are high. As an alternative to insecticide control, ARS researchers in Fort Pierce, FL, are developing a control strategy called “Conservation Biological Control” in which certain plants are grown to support insect predators that attack the psyllid. The scientists demonstrated that a statistical method called Response Surface Methodology could be used to optimize mixtures of plants to support the insect predators of ACP. Various plants were used, including mixtures of crown-of-thorns, lima beans, wild poinsettia, flowering buckwheat, partridge pea, and ornamental portulaca. Results showed that when formulating a plant mixture to aid in biological control of the psyllid, consideration should be given to the proportion of each plant species included in the mixture. (NP304, C3, PS3c, Project No. 6036-22000-030-00D)

Assembly and annotation of the brown marmorated stink bug genome. ARS scientists in Beltsville, MD, led an international consortium of 57 scientists belonging to 31 institutions to sequence, assemble, and annotate (determine the genes) the genome of the brown marmorated stink bug. The stink bug is a highly mobile worldwide invasive pest that can attack a wide range of important crops and causes particularly serious damage to fruit crops. The researchers found an expanded set of genes encoding taste receptors, as well as genes involved in detoxification and digestion, which are probably key to the extreme breadth of the stink bug’s dietary preferences. Researchers will use the gene sequences and their annotations to guide genetic knockdown or knockout experiments that will in turn allow the development of RNA interference-based biopesticides, or other control strategies, that are more specific for the stink bug than conventional insecticides. (NP304, C3, PS3a, Project No. 8042-22000-291-00D)

Identification of peptides for control of the insect vector of citrus greening. ARS scientists in Ithaca, NY, published a comprehensive description of the peptidome of the vector of HLB (Huanglongbing, aka citrus greening), the Asian citrus psyllid (Diaphorina citri), which included hundreds of molecules with classic signatures of biologically active peptides. This data collection is a rich resource for the development of novel antimicrobials, and for more than 100 candidate insect neuropeptides. This work led to an APHIS-funded HLB initiative to screen a series of these neuropeptides, some of which have demonstrated insecticidal activity against other hemipteran insects, as new and environmentally friendly pesticides for the management of the psyllid in citrus. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

Grasshoppers affect carbon sequestration. Together with collaborators, ARS researchers in Sidney, MT, found that the food preferences of aboveground and belowground grasshopper communities affect soil composition. Grasshopper diet shifted soil community composition and function, while grasshopper composition changed only belowground functions. The results demonstrate that grasshopper feeding patterns may be important for understanding how grasshoppers affect both plants and belowground organisms. In a novel finding, grasshopper
presence increased carbon storage belowground, which is a previously underappreciated pathway of soil carbon sequestration. This study demonstrates that grasshoppers can affect belowground parts of rangeland systems, which could influence livestock grazing on rangeland during grasshopper outbreaks. (NP304, C3, PS3b and PS3d, Project No. 3032-22000-018-00D)

**Component 4 – Protection of Postharvest Commodities, Quarantine, and Methyl Bromide Alternatives**

New methods to control invasive horticultural pests and pests of quarantine importance. Alternatives to methyl bromide fumigation are urgently needed for treating economically important insect pests of harvested fruit. ARS scientists in Parlier, CA, developed a novel postharvest fumigation protocol to control codling moth that were infesting shelled walnuts packed in new fiberboard cartons. U.S. exports of shelled walnuts to Japan are valued at $80 million annually. A quarantine protocol for trailers containing citrus using postharvest fogging was developed against Asian citrus psyllid. A fumigation protocol using phosphine was developed against bean thrips to retain U.S. market access for fresh citrus to New Zealand, valued at $20 million annually. Phosphine fumigation protocols were also developed to control black widow spider and spotted wing drosophila to retain U.S. market access for table grapes to Australia valued at of $180 million annually. This ARS research directly resulted in market retention or expansion and served as the basis for interaction between industry, USDA Foreign Agricultural Service, USDA Animal and Plant Health Inspection Service, and respective counterparts in other countries. (NP304, C4, PS4a, Project No. 2034-43000-040-00D)

Genes identified in insects that infest stored food products. Understanding the factors that allow insects to colonize different types of stored products will ultimately lead to tactics that can reduce and prevent infestations. Using recently compiled insect genomes from Ag100Pest, the effort to sequence the top 100 agricultural pests in the United States, ARS scientists in Manhattan, KS, in conjunction with researchers from University of Memphis, Indiana University, and several other research institutions have discovered rapidly evolving gene families that allow insects to adapt to new food sources. These gene families include those involved in the ability to perceive and respond to volatiles from food resources as well as those involved in the ability to digest complex carbohydrates present in plant cell walls and starchy foods. The ability to make long-read sequencing libraries from small amounts of insect tissue has greatly expanded the number of insect genomes scientists now have available to them and will lead to further insights into genetic adaptations that have allowed diverse lineages of insects to exploit stored commodities. (NP304, C4, PS4b, Project No. 3020-43000-032-00D)

Nitric oxide fumigation controls navel orange worm in walnut. The navel orange worm (NOW) causes postharvest losses to stored tree nuts. Nitric oxide (NO), a fumigant used for postharvest pest control, was evaluated by ARS researchers in Salinas, CA, for its efficacy against eggs, larvae, and pupae of NOW. Susceptibility to NO fumigation varied among different life stages with NOW eggs being most tolerant, followed by pupae and then larvae. Treatment time needed for complete control of NOW decreased with increasing NO concentrations, and complete control of NOW eggs was achieved in 16-hour fumigation with 2 percent NO. The
study demonstrated that NO fumigation was effective against NOW on walnut and has potential to be an alternative treatment for postharvest control of this pest of tree nuts. (NP304, C4, PS4b, Project No. 2038-22430-002-00D)

Optimizing fumigations for increased efficacy and human and environmental health. Having data on worker and consumer exposure to fumigants is essential to ensure human health and expand agricultural productivity. ARS researchers in Parlier, CA, studied the effects of ethyl formate exposure and generated and submitted data to support a Section 3 registration of its use to control Asian citrus psyllid. Also, they submitted worker and consumer exposure data to support a Section 3 re-registration of the use of sulfur dioxide to treat table grape and blueberry exports. Furthermore, the researchers submitted worker and consumer exposure data to support a registration (through Sections 18 and 24c) of sulfur dioxide-releasing sodium metabisulfite products for use on blueberry exports. American farmers export hundreds of millions of dollars in blueberry and table grape exports per year. The data on these products served as the basis for ARS interaction with industry, U.S. Environmental Protection Agency, and state government representatives. (NP304, C4, PS4a, Project No. 2034-43000-040-00D)

Quarantine irradiation treatment for hitchhiking ants. Ants can be problematic hitchhiker pests in exported fruits and vegetables that cause rejection or return shipment. ARS researchers in Hilo, HI, conducted irradiation tolerance studies with several different species of ants and concluded that the generic irradiation dose commonly used against fruit flies also controls hitchhiking ants. The findings were provided to regulatory officials at the Animal and Plant Health Inspection Service and California Department of Agriculture. Results of the research were also submitted to the United Nations International Plant Protection Convention for consideration as a phytosanitary treatment. (NP304, C4, PS4a, Project No. 2040-43000-017-00D)

Quarantine treatment for fresh apple cultivars. Disinfestation of fresh commodities with X-ray radiation is an effective and accepted quarantine treatment for export markets. ARS scientists in Hilo, HI, in collaboration with New Zealand Institute of Plant and Food Research colleagues, determined the tolerance of four apple varieties to ionizing radiation in a commercial facility. All cultivars but ‘Royal Gala’ maintained quality following treatment at doses that were effective for controlling codling moth and other major insect pests. The results will support near-term adoption of X-ray quarantine treatment by the New Zealand apple industry and future use of this technology by the U.S. apple industry. (NP304, C4, PS4a, Project No. 2040-43000-017-00D)

Kairomone-based lure for cocoa pod borer developed. Cocoa pod borer is the most serious insect pest impacting cacao production in Indonesia, the world’s third-largest producer of cacao. Pest detection currently relies on a synthetic pheromone lure that is attractive to male moths, but the lures are expensive and products from different sources are highly variable in efficacy. Under a Cooperative Research and Development Agreement with Mars Inc., ARS researchers in Miami, FL, conducted laboratory and field evaluations of various plant essential oils and fruit extracts, and a male-specific kairomone-based attractant was discovered.
Subsequent research optimized the dose, formulation, and other modifications to develop a kairomone lure that performs as well or better than the pheromone lure. Moreover, the kairomone lure has a significantly longer field life and costs much less to produce than the pheromone lure. A patent application has been submitted for this invention. This improved lure will directly benefit the cocoa industry and indirectly benefit U.S. sugar, dairy, and nut industries that partner in the production of chocolate. (NP304, C4, PS4a, Project No. 6038-22000-006-00)

Molecular profiling of citrus leaves: a new tool for early detection of citrus greening disease. Citrus greening is devastating orchards in Florida and the south, and threatens groves in California. A collaboration between ARS scientists in Ithaca, NY, and Riverside, CA, identified molecular changes in citrus leaves resulting from infection with the causative citrus greening bacterium. Leaf samples were collected biweekly from lemon and navel orange trees for a year following graft inoculation with either pathogen-containing or healthy budwood and analyzed for biomarkers of infection. RNA, protein, and metabolite levels were compared over time between healthy and infected plants, revealing molecular profiles that were associated with infection months before visual symptoms of disease appeared. Results from this study reveal differences in the response to infection between these two distinct varieties of citrus and provide insight into how the plant response to the pathogen changes with time. These findings can be used to improve diagnostic tests for citrus greening disease. This high-profile research was featured on the cover of a 2020 issue of *Journal of Proteome Research*. (NP304, C1, PS1a, PS1c; C3, PS3a, PS3b, PS3c, PS3d; C4, PS4a, PS4b, Project No. 8062-22410-006-00D)

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