United States Department of Agriculture
Agricultural Research Service

National Program 304 • Crop Protection and Quarantine

FY 2021 Annual Report

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 2021. 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 2021 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 Steve Young (steve.young@usda.gov).

Component 1 – Systematics and Identification

Identifying very small biocontrol organisms with short DNA sections. DNA barcoding can be defined as the use of a short section of DNA from a specific gene of an organism. Specific gene regions are chosen because they have less variation within a species and thus can lead to more accurate identification of the organism. Current techniques require DNA extraction that is time consuming, costly, and can result in DNA loss, which can be particularly problematic when studying very small insects and mites. When pooling multiple insects and mites for extraction, a failure in detecting different species can occur as small insects are mixed during the sampling. ARS scientists are developing a DNA barcoding protocol to improve detection efficacy and sensitivity to overcome DNA loss. At the ARS European Biological Control Laboratory in Montpellier, France, scientists used modified Polymerase Chain Reaction to amplify the DNA of individual eriophyid mites and were able to amplify the barcode region of an individual mite that is being considered as a biological control agent for the tree of heaven, a highly invasive tree that is spreading in the eastern United States. This protocol greatly reduces the chances of amplifying different DNAs from a mixed sample because it provides more genetic material for analysis. (NP304, C1, PS1A, Project No. 0212-22000-031-00D)

A bacteria-based biopesticide controls pecan weevil and preserves natural enemies. The pecan weevil is a major pest of pecans and is typically controlled with chemical insecticides. However, these insecticides may be harmful to humans and the environment, kill beneficial natural enemies such as lady beetles, and boost numbers of pecan aphids, another group of major pecan pests. An environmentally safe biopesticide, “Grandevo”, based on a naturally occurring bacterium that was discovered by ARS scientists in Beltsville, Maryland, was used by ARS scientists in Byron, Georgia. It produced equal levels of pecan weevil control when compared with commonly used chemical insecticides. Furthermore, the biopesticide contributed to pecan aphid control and did not harm beneficial natural enemies during field experiments. ARS scientists determined the bacteria-based biopesticides are a viable, eco-friendly tool for the control of pecan weevils in both organic and non-organic production systems. (NP304, C1, PS1A, Project No. 6042-22000-024-00D)

Nanoparticle formulations enhance biopesticide efficacy. Environmentally friendly biopesticides such as entomopathogenic (insect-killing) fungi can control various economically important insect pests such as pecan weevils. The efficacy of these biopesticides, however, can be limited due to their sensitivity to ultraviolet radiation, so it is critical to develop new formulations that protect the biopesticide organisms from environmental stress. ARS researchers in Byron, Georgia, and Israeli partners, discovered that nanoparticle-based formulations protect biopesticides from ultraviolet radiation and thereby increase pest control efficacy. This technology could potentially lead to improved sustainability in pest management practices. (NP304, C1, PS1A, Project No. 6042-22000-024-00D)

Rearing methods and phytosanitary irradiation treatments to control a slug associated with rat lungworm disease. Hawaii island is a hot spot for rat lungworm disease in humans, a serious and sometimes fatal disease caused by the nematode *Angiostrongylus cantonensis*. The nematode, endemic to Southeast Asia and Pacific Islands, has recently been introduced in Hawaii and causes occasional cases in the United States mainland. Rats serve as reservoir for this nematode, which can then be spread to fresh produce by snails and slugs, and ultimately to humans. The primary mode of infection in Hawaii is by accidental ingesting of a slug, named *Parmarion martensi*, that carries the nematode. The slug has been found on sweet potatoes, fruits, and vegetables exported from Hawaii to the continental United States. ARS researchers in Hilo, Hawaii, and University of Hawaii scientists have developed a method to maintain slug colonies in the laboratory and rear them for experimental purposes. Rearing is an important first step for developing phytosanitary methods to eliminate the slug and nematode from produce. Additionally, researchers demonstrated that X-ray radiation reduced feeding and survival, and prevented slug reproduction. These research findings will increase safety of produce grown in regions with rat lungworm and reduce cases of eosinophilic meningitis in humans. (NP304, C1, PS 1A, Project No. 2040-43000-018-00D)

Novel intercropping increases beneficial insects. In the Northern Great Plains, wheat is commonly rotated with fallow and growers are interested in incorporating *Brassica carinata* and *Camelina sativa*, two potential biofuel crops, into their systems. The impacts of these crops on honeybees and other beneficial insects are unknown. ARS researchers in Sidney, Montana, documented 45 native bee species, honeybees, and 24 genera of beneficial wasps utilizing *B. carinata* and *C. sativa* in a wheat-fallow system. The additional benefit of these crops to support a wide variety of beneficial insects could be an added incentive for adoption by growers. (NP304, C1, C3, PS1A, PS3A, Project No. 3032-21220-003-00D)

Promoters for producing genetically modified fruit fly pests for enhanced Sterile Insect Technique (SIT). Enhanced SIT for fruit fly pests could substantially reduce costs and yield losses in fruit and vegetable agriculture. Genetic modification allows the creation of fly lines that can potentially make SIT far more efficient and effective. Scientists in Gainesville, Florida, isolated and identified promoters with the potential to turn on lethality-causing genes in early embryos and gametes. These have the potential to overcome several technical problems that have reduced SIT efficiency, resulting in more highly efficient lethality systems for use in SIT for multiple fruit fly pest species. (NP304, C1, PS1B, Project No. 6036-22000-034-00D)

Phylogenomics of the armyworms. The noctuid genus *Spodoptera* includes species that pose some of the world's greatest threats to agriculture, among them the fall armyworm, which has crossed and overtaken three continents in the last 5 years. ARS researchers at the Smithsonian Institution's National Museum of Natural History and collaborators from France, Kenya, United Kingdom, and Australia, used genome skimming to generate mitogenomic (mitochondrial genome) data for 14 species and combined these with nuclear data to produce a backbone phylogeny for the genus and an updated phylogenetic framework for the genus. Their analyses recovered two new ecologically diverse clades that correspond to larval feeding morphology. Dating analyses indicated a more recent origin than previously thought for the *Spodoptera* genus. (NP304, C1, PS1A, Project No. 8042-22000-314-00D)

Improving identification tools for fruit fly pests. True fruit flies include some of the most important pests of commercial and subsistence fruit crops, including citrus, mango, peach, apple, and many others. Many species are invasive and threaten U.S. agriculture, including multiple species of *Anastrepha*, the largest and most economically important genus in the U.S. tropics and subtropics. Current taxonomy is based mainly on the adult female; the immature stages, which actually damage the fruit, and males of many species cannot be reliably identified. ARS scientists in Washington, D.C., collaborated with the Florida Department of Agriculture and Consumer Services, University of Florida, North Carolina State University, and APHIS-CPHST on improving fruit fly identification. The ARS scientists generated or verified and analyzed a large library of specific DNA barcoding sequences for 260 species of *Anastrepha*, indicating that the majority of species can be distinguished with this information. However, some closely related pest species cannot be diagnosed with this method, indicating additional investigation is needed. This new information is already being used by APHIS and other regulatory agencies and is critical for more rapid and reliable identification of all life stages of invasive fruit fly species. (NP304, C1, PS1A, Project No. 8042-22310-001-00D)

New entomopathogenic fungi associated with emerald ash borer. The emerald ash borer (EAB), *Agrilus planipennis*, is an invasive insect pest from Asia that is killing ash trees across North America. EAB has caused substantial economic losses to wood related industries and homeowners, municipalities, and state and national forests. Other invasive wood-boring beetles are known to spread microbes, particularly fungi, that may cause tree disease, but little is known about the microbial communities associated with EAB. ARS scientists in Ithaca, New York, and University of Minnesota collaborators investigated fungi associated with EAB. This research uncovered many plant disease-causing fungi (soft rot and canker) and 93 insect-infecting fungi, referred to as entomopathogenic fungi, with potential as biocontrol agents. The insect-infecting fungi expand the arsenal of tools available to ARS and its stakeholders to fight the EAB and, potentially, other serious pests. (NP304, C1 and C3, PS3a and 3a, Project No. 8062-224-007-00D)

Biocontrol agent testing and approval for federally listed noxious weed. Rangelands and grasslands comprise 30 percent of land cover in the United States and are challenging environments in which to manage invasive weeds; costs are estimated at $6 billion annually. Common crupina (*Crupina vulgaris*) infests thousands of hectares of grasslands in Idaho, California, Washington, and Oregon. Common crupina degrades native and beneficial plant communities and reduces rangeland forage productivity. ARS researchers in Fort Detrick, Maryland, conducted studies and performed regulatory reviews on the host-specific fungal pathogen *Ramularia crupinae* for common crupina. Field releases have been approved and ongoing studies are being conducted in the western United States. By combining the biocontrol pathogen with other tactics, rangelands will benefit from reduced populations of common crupina. (NP304, C1, PS1C, Project No. 8044-22000-047-00D)

Importance of native range of alien invasive plants. Flowering rush is an aquatic weed from Eurasia and is the focus of a multimillion-dollar eradication and management program. In the western United States, the common genotype of flowering rush is reported to be resistant to biological control agents. ARS researchers in Sidney, Montana, determined that the plants invading the Pacific Northwest originated from the Overijssel Province of the Netherlands. This information will allow researchers to more efficiently explore for co-evolved biological control agents that can be used in the United States. (NP304, C1, C2, PS1B, PS2B, Project No. 3032-21220-003-00D)

Component 2 – Weeds

Characterization of a replicon in the development of herbicide-resistant weeds. Across midwestern and southeastern U.S. agronomic systems, Palmer amaranth has become known as one of the most difficult weeds to control. Current management tactics that employ herbicides have proven to be of little use because Palmer amaranth has become resistant to chemical treatment and is lowering crop yields. ARS scientists in Stoneville, Mississippi, and Clemson University researchers conducted groundbreaking research on the replicon, the molecular mechanism that confers glyphosate herbicide resistance in Palmer amaranth, and identified genetic elements to encode the entire replicon in resistant plants. These results will allow scientists to better understand the molecular mechanics that are involved with resistance development and potentially apply this information to other systems, such as cancer in humans. (NP304, C2, PS2A, Project No. 6066-21000-061-00D)

New biological control agent for the weed earleaf Acacia. Biological control agents are urgently required for the earleaf Acacia tree, which is native to Australia and is now spreading in Florida. If left uncontrolled, this invasive tree could rapidly expand, reducing biodiversity by invading protected areas such as the Everglades National Park. Scientists in the ARS-associated laboratory in Brisbane, Australia, evaluated a damaging gall-forming wasp as a biological control agent against earleaf Acacia, since closely related species have successfully controlled Acacia species in Africa. After successful colonization in the lab, preliminary host range testing determined that this wasp is highly specific and therefore safe for export, so it can be sent to U.S. quarantine for final host-range safety and efficacy testing. (NP304, C2, PS2A, Project No. 0206-22000-003-00D)

Characterization of a replicon that causes a problematic weed to resist herbicides. Palmer amaranth is one of the most difficult weeds to control in agronomic systems across the Midwest and Southeast United States. Management tactics that rely on herbicides are of little use, since the weed has developed resistance to multiple modes of action. This resistance has reduced lower yields. ARS researchers in Stoneville, Mississippi, and Clemson University conducted groundbreaking research on the replicon in Palmer amaranth, the entity that confers resistance to the herbicide glyphosate. A new discovery has revealed the presence of enough miniature, circular, genetic elements to encode the entire replicon in resistant plants. This new finding will help detail the molecular mechanism involved in resistance and could have applications to other systems, such as cancer in humans. (NP304, C2, PS2A, Project No. 6066-21000-061-00D)

Critical habitat restoration reduces fuel loads and wildfire risks and increases forage availability. Invasive annual grasses have caused millions of acres of damage to rangelands in the western United States through displacing native species and generating excessive biomass that fuels wildfires. ARS scientists in Reno, Nevada, tested the efficacy of transplanting Wyoming big sagebrush and antelope bitterbrush and direct seeding of Wyoming big sagebrush and antelope bitterbrush with perennial grasses to evaluate strategies for their establishment, assess their effectiveness at suppressing invasive annual grasses and associated fuel loads, and improve grazing resources in the western U.S. rangelands. Transplanting was the most successful method in establishing Wyoming big sagebrush, while direct seeding was more beneficial to antelope bitterbrush. Seeding perennial grasses at proper seeding rates did not limit shrub establishment and reduced cheatgrass fuel loads. Using proper restoration methodologies, fall transplanting and rangeland drill seeding increased critical browse species by more than 400 percent and perennial grass densities by more than 600 percent. At the same time these methods reduced cheatgrass densities and associated fuels by more than 85 percent, therefore providing sustainable grazing and wildlife resources. (NP304, C2, PS2B, Project No. 2060-22000-025-00D)

Cereal rye cover crop plays important role in weed management for alternative crops. Alternative vegetable crops—such as edamame, lima bean, and snap bean—are increasingly being grown for processing, but weeds are a major production problem, and there are few cost-effective tools to manage them. ARS researchers in Urbana, Illinois, examined the role of early-terminated cereal rye cover crop on weed suppression and vegetable crop yield. The system selectively enhanced weed control in edamame and was comparable to hand weeding. A new, economically viable solution to weed management now exists that lessens the reliance on herbicides for edamame. The expanding market in the United States for alternative vegetable crops such as edamame supports the development of a domestic industry that is both competitive and sustainable. (NP304, C2, PS2A, Project No. 5012-12220-010-00D)

Including tiller buds with grass seeds may enhance restoration of sagebrush steppe ecosystems. The sagebrush steppe ecosystem is in serious need of restoration and seed-based seeding has not provided an adequate emergence of native grasses. ARS research in Burns, Oregon, indicated that new plants initiated from tiller buds emerged in half the time as new plants developing from seeds at low temperatures (7 degrees C). This accelerated emergence persisted at warmer temperatures, though to a lesser extent. After 21 days, emergence was 8 times greater from tillers than from seed for bottlebrush squirreltail and 10 times greater for Sandberg bluegrass, two essential native grass species. Accelerated emergence could allow for greater restoration success in critical but rugged ecosystems. (NP304, C2, PS2B, Project No. 2070-22000-007-00D)

Cape-ivy biocontrol affected by simulated drought. Cape-ivy is a vine-like plant that has invaded streambanks, forests, and scrub/bluff habitats in coastal California ecosystems, consuming water and displacing rare native species. ARS researchers in Albany, California, released a fly that makes tumors, or galls, in shoot tips, but found that fly populations did not establish at warmer sites. In greenhouse trials, fly females laid 72 percent fewer eggs on plants subjected to simulated drought. Fifty percent fewer galls were produced, galls were 39 percent smaller, and the developing flies inside were 35 percent lighter. Adult reproduction was 78 percent lower under drought than on well-watered plants, and time to complete development was one week longer. The results provide important information to researchers and natural resource agencies implementing biological control of Cape-ivy about when to release the fly in natural areas that are under increasing drought stress due to climate change. (NP304, C2, PS2B, Project No. 2030-22000-031-00D)

Pre-emergent herbicides and seeding reduce fuel loads and risk of wildfire. Invasive annual grasses cause damage to millions of acres of rangelands in the western United States by displacing native species and generating excessive biomass that contributes to wildfires. ARS scientists in Reno, Nevada, in cooperation with local, state, and federal partners, initiated an integrated approach of using pre-emergent herbicides to control cheatgrass and other annual weeds along with seeding methodologies of desirable perennial species to restore critical grazing and wildlife resources. These tactics, in combination with proper seed mixes, resulted in high success rates for perennial grasses, shrubs, and forbs. Perennial grass densities from seeding operations increased four-fold while cheatgrass densities and associated fuels have declined by more than 80 percent. Aerial seeding of Wyoming big sagebrush and western yarrow on selected habitats resulted in high levels of establishment, thus supporting sage grouse, mule deer, and other wildlife species. (NP304, C2, PS2B, Project No. 2060-22000-025-000D)

Fire as a management tool for woody rangeland plants. Woody plant encroachment currently affects more than one quarter of all western rangelands, costing producers more than $5 billion during the past 30 years. ARS researchers in Sidney, Montana, assessed the effectiveness of fire for the regional management of juniper species from 1999 to 2017. Fire reduced creeping juniper densities, but recovery occurred within 20 years and plant community compositional shifts were short-lived. Producers are now equipped with critical information on the use of fire for managing creeping juniper and the long- and short-term trade-offs. (NP304, C2, PS2A, PS2C, Project No. 3032-21220-003-00D)

Release of the air potato beetle (*Lilioceris egena*). Air potato (*Dioscorea bulbifera*) is a subtropical Old-World vine that has become invasive along the Gulf Coast, especially Florida. ARS researchers in Fort Lauderdale, Florida, received a permit to release a beetle known as *Lilioceris egena* in the United States, having shown that this beetle is a specialist on the reproductive bulbils, or potatoes, associated with air potato. Despite slowed growth and bulbil production caused by a previously released Asian beetle (*Lilioceris cheni*), air potato is still spreading. By attacking air potato bulbils, *L. egena* will significantly impede population regrowth and allow native species to reclaim land currently covered by air potato. (NP304, C2, PS2B, Project No. 6032-22000-013-00D)

Release of a new biological control agent targeting yellow starthistle. Yellow starthistle is a spiny rangeland weed that is toxic to horses and has invaded about 20 million acres in the western United States. ARS researchers in Albany, California, obtained approval to release the rosette-feeding weevil (*Ceratapion basicorne*), the first new biological agent released against this weed in almost 20 years, and the first that feeds on roots and rosettes of immature plants. Weevils obtained from Greece with the help of foreign collaborators were multiplied in the laboratory and released in the field in California. Weevils were provided to state cooperators in Colorado and Idaho for them to breed for release. This weevil is expected to increase and spread on its own, which will help reduce yellow starthistle infestations. Successful biological control will increase forage production and desirable native vegetation and will reduce herbicide application. (NP304, C2, PS2B, Project No. 2030-22000-031-00D)

New biological control agent for Chinese tallow. Chinese tallow is an invasive tree infesting thousands of acres in the southeastern United States. Host range testing indicated the flea beetle (*Bikasha collaris)* and the moth *Gadirtha fusca* will be safe for release. This release was recommended by Technical Advisory Group (APHIS) and U.S. Fish & Wildlife Service. ARS scientists in Fort Lauderdale, Florida, are waiting for the issuance of a release permit from USDA-APHIS. When the release is approved, these biological control agents could provide land managers and farmers with a cost-effective means of controlling Chinese tallow by reducing the current reliance on herbicidal control. (NP304, C2, PS2B, Project No. 6032-22000-013-00D)

Impacts of fire on mesquite persistence. Mesquite encroachment into arid lands worldwide has negatively impacted livelihoods and ecosystems. In Texas, more than $50 million has been spent in 10 years to control mesquite. ARS researchers in Sidney, Montana, and university collaborators assessed mesquite persistence across a range of fire intensities. High-intensity fire caused mortality in 29 percent of mesquite, but did not alter soil chemistry or microbes, and did not permanently harm dominant grass species. High intensity fires are a low-cost option as part of an overall management program for reducing mesquite abundance in rangelands. (NP304, C2, PS2A, PS2C, Project No. 3032-21220-003-00D)

Quantitative assessment of leafy spurge biocontrol. Leafy spurge continues to be a problematic weed for rangeland producers and managers despite a widely successful biological control program. ARS researchers in Sidney, Montana, and university collaborators surveyed leafy spurge and biological control agent populations across Montana, North Dakota, and Idaho. The presence of biological control agents was not positively correlated with leafy spurge populations. Further research is needed to develop integrated weed management approaches that augment biological control to better limit current and new leafy spurge infestations. (NP304, C2, PS2B, Project No. 3032-21220-003-00D)

Bioherbicides to control weeds. Phytopathogenic fungi have potential for use as bioherbicides on weeds and invasive plants. ARS researchers in Stoneville, Mississippi, conducted studies on preparations of the fungus *Myrothecium verrucaria* (Mv) and a recently discovered sector (Mv-Sector BSH) of the fungus. Applications of Mv and Mv-Sector BSH preparations to hemp sesbania, sicklepod, kudzu, and glyphosate-resistant Palmer amaranth showed comparable results between sector and Mv efficacy. The development of commercially viable microbial herbicides could provide safe, cost-effective, and non-chemical control tools for use in agriculture, chemically sensitive environments, and natural ecosystems. (NP304, C2, PS2B, Project No. 5012-12220-010-00D)

Biocontrol of problematic invasive aquatic weeds. Common duckweed (*Lemna minor*) is a rapidly spreading aquatic plant that deprives ponds of oxygen and leads to the death of fish and beneficial algae. ARS researchers in Stoneville, Mississippi, conducted experiments using a fungal formulation and found that 95-100 percent mortality of duckweed was achieved within 48 hours following inoculation. There is potential for controlling an important invasive weed with naturally occurring fungal compounds and indirectly inhibiting the production of cyanobacteria. (NP304, C2, PS2B, Project No. 5012-12220-010-00D)

Formulations to enhance bioherbicidal pathogen efficacy. Eastern black nightshade (*Solanum ptycanthum*) is a problematic weed found throughout the eastern United States. ARS researchers in Stoneville, Mississippi, compared two formulations of a bioherbicidal fungus, *Colletotrichum coccodes*, for controlling nightshade. In the field, nightshade control rates of more than 90 percent were achieved with the invert emulsion (water in oil) formulation, demonstrating its potential as a bioherbicide. Additional studies are needed under a variety of growth and environmental conditions. (NP304, C2, PS2B, Project No. 5012-12220-010-00D)

Foundation established for Great Basin and sagebrush biome ecological research. The sagebrush biome stretches from Washington State through the Great Basin and across to the Dakotas and northern New Mexico. Ecological degradation in this vast area has affected more than 350 plant and animal species. ARS researchers in Reno, Nevada, published seminal documents that provide the foundation for future ecological research. The first is an ARS-led synthesis of all climate change research to date in the Great Basin, which covers approximately 6 percent of the land area of the continental United States. Only 2 years after publication, this 2019 article has been cited more than 25 times in studies of wildlife, stream health, adaptive environmental management, erosion, and other fields. The second document is a multi-agency report detailing best practices for restoration in the sagebrush biome of the western United States. These two documents represent a new and invaluable resource for land managers, stakeholders, policymakers, and the research community. (NP304, C2, PS2B, Project No. 2060-22000-025-00D)

New areawide project on integrated pest management of waterhyacinth. Initial studies continue to provide strong evidence for the efficacy of integrated management of waterhyacinth (*Pontederia crassipes*): when insects for biocontrol are present, reduced herbicide rates and increased time intervals yield the same results as higher inputs. ARS researchers in Fort Lauderdale, Florida, received funding for a project that will elucidate potential new management practices utilizing the full suite of available biological control insects, integrated with other control measures. Resources from this new award will also explore the viability of *Thrypticus* spp. for use as biological control agents. Flies in this genus are closely related and often cryptic, confusing initial workers. Researchers at the Fundacíon para el Estudio de Especies Invasivas (FuEDEI) in Argentina found definitive information for differentiating these species and now have pure lines of a species of *Thrypticus* believed to specialize on *P. crassipes*. (NP304, C2, PS2B, Project No. 6032-22000-013-00D)

Cover crop residue decomposition and nutrient release in conservation tillage systems. Growers need site-specific knowledge on how cover crop surface residues decompose and release nutrients. ARS scientists in Beltsville, Maryland, conducted studies examining the effects of climate on cover crop residue decomposition and nutrient release. Moisture proved to be more important than temperature in driving cover crop residue decomposition. Additional research is needed to address nutrients. Ultimately, studies will lead to improved adaptive nitrogen calculators used by researchers, farmers, and policymakers. (NP304, C2, PS2A, Project No. 8042-22000-167-00D)

Minimizing seed banks with harvest weed control. Harvest weed seed control is a promising nonchemical weed management strategy that involves the destruction of weed seeds during crop harvest. ARS scientists in Beltsville, Maryland, in collaboration with a national team of U.S. universities, demonstrated which weed species in the United States are more susceptible to harvest control. This work will help growers dealing with numerous weeds, including those resistant to herbicides. (NP304, C2, PS2A, Project No. 8042-22000-167-00D)

Component 3 – Insects and Mites

Modifying plant traits without modifying plant genes. Insect-vectored plant disease can be extremely difficult to control, especially in long-lived trees, because a single piercing-sucking insect can inject a disease-causing dose of a pathogen. ARS researchers in Fort Pierce, Florida, in collaboration with ARS researchers in Ithaca, New York, and a small agribusiness in Florida, developed a novel Agrobacterium-based method of creating a host plant cell that can produce its own growth regulating molecules and can be grown as symbionts attached to crop plants. This method was first demonstrated in tomato and sunflower plants and results showed that symbionts engineered to produce *Bacillus thuringiensis* (Bt) toxins killed 100 percent of caterpillar larvae after 3 days of feeding on an artificial diet supplemented with engineered symbiont tissue. Insects with chewing mouthparts, such as caterpillars, were specifically chosen to prove that the symbiont produces a lethal amount of Bt toxin. The next steps of the study will be to test the engineered symbiont tissue on piercing-sucking insects, such as the Asian citrus psyllid, which is a vector of citrus greening disease. Implementing this pest-insect control strategy will enable a rapid response to emerging pest-insect threats and will more easily deliver therapeutic technologies to diverse germplasms. Researchers are also planning to test if engineered symbiont tissues can be used as a biofactory of harvestable biological insecticide molecules or therapeutics that could potentially create new cropping systems. Results of this study will show that symbiont technology has the potential to bring transformational advancements to plant disease management and the mass production of biomolecules. (NP304, C3, PS3B and 3C, Project Nos. 6034-22320-007-00D and 8062-22410-007-00D)

Effective management of soybean aphid without insecticides**.** Soybean aphids are a significant threat to the profitability of soybean production in the midwestern United States and southern Canada. One strategy to manage soybean aphids is through a combination of insecticide-treated seeds and late-season foliar insecticide treatments, but this strategy increases productions costs, inadvertently selects for insecticide-resistant aphids, and increases the risk of human exposure to insecticides. ARS scientists in Brookings, South Dakota, conducted a field study to test the effectiveness of resistant cultivars against insecticide seed treatment, foliar insecticide spray, and an aphid-resistant soybean cultivar. While each tactic independently reduced the population densities of soybean aphids, the use of the resistant cultivar produced the greatest impacts on aphids and reduced their populations by 28- to 150-fold per year. In contrast, insecticide seed treatments reduced aphid populations by 1.7- to 3.5-folder per year and foliar insecticide sprays reduced aphid populations by 2.0- to 5.6-fold per year. These findings demonstrated the superior effectiveness of using soybean cultivars with aphid-resistant genes. (NP304, C3, PS3C, Project No. 3080-21220-007-00D)

Transgenic cotton and sterile insect releases synergize eradication of pink bollworm from the United States. The pink bollworm is one of the world's most invasive insects and has been a major pest of cotton in the United States since 1917. However, decades of effort and implementation of the Binational Pink Bollworm Eradication Program culminated in the USDA Secretary officially declaring the pink bollworm eradicated from the cotton-growing regions of the continental United States in 2018. ARS researchers in Maricopa, Arizona, collaborated with the USDA Animal and Plant Health Inspection Service, Arizona Cotton Research and Protection Council, and University of Arizona on using models to demonstrate that eradication was made possible by the synergistic interaction of *Bacillus thuringiensis* cotton and sterile insect releases. Researchers determined that eradication saved cotton growers in the United States $224 million from 2014 to 2020 and was associated with an 82 percent reduction in insecticide use for all cotton pests in Arizona during this same period. The economic and social benefits achieved have wide sweeping impacts on agriculture and society and demonstrate the benefits of using agricultural biotechnology in concert with classical pest control tactics. (NP304, C3, PS3B, Project No. 2020-22620-023-00D)

<u>Model predicts threat of zebra chip potato disease</u>. In the U.S. Pacific Northwest, where most of the U.S. potato crop is grown, zebra chip has become a widespread and economically devastating potato disease. The zebra chip pathogen is a bacterium that is spread by the potato psyllid and proper pathogenic control can be accomplished only by controlling the insect vector. U.S. potato farmers have had difficulty controlling zebra chip because they lack the tools to estimate potato psyllids populations, which can fluctuate greatly from year to year. ARS scientists in Wapato, Washington, and Washington State University collaborators determined that the weed matrimony vine is an important host plant for potato psyllids in early spring. Matrimony vine was not found to be susceptible to the zebra chip pathogen, and is therefore not the source of infected psyllids, but ARS scientists found that psyllid numbers on matrimony vines in the spring can be used as an indicator of psyllid populations in potatoes in late August. This novel forecasting method for psyllid populations will allow growers to take action to protect their crop and associated revenues in years when psyllid outbreaks are expected. (NP304, C3, PS3B, Project No. 2092-22000-022-00D)

<u>Assembly of the red-banded stink bug genome</u>. The red-banded stink bug (RBSB) *Piezodorus guildinii* is major soybean pest and uncontrolled outbreaks can cause significant economic damage to soybeans from early seed development stages to mature seeds that are ready to harvest. ARS researchers in Stoneville, Mississippi, sequenced the RBSB genome to obtain a high-quality assembly. The sequencing and assembly of the RBSB genome will allow researchers to identify the genetic basis of insecticide resistance, genetic diversity, gene flow, migration, and population structure of this insect pest. (NP304, C3, PS3B, Project No. 6066-22000-090-00D)

<u>Gut content of brown marmorated stink bug</u>. The invasive brown marmorated stink bug recently arrived in the U.S. Pacific Northwest, where most U.S. tree fruit is grown, and is causing a significant economic impact to orchards. Managing the brown marmorated stink bug has been difficult because non-crop sources of stink bugs entering orchards have been hard to determine. ARS researchers in Wapato, Washington, and Washington State University scientists developed a PCR-based method to detect plant DNA in the guts of the brown marmorated stink bug. This will enable researchers to determine which non-crop plants the stink bugs fed upon prior their capture in orchards. This information can be used to develop effective areawide management approaches for this pest and reduce their economic impact on orchards. (NP304, C3, PS3B, Project No. 2092-22430-003-00D)

Variable weather threatens future efficacy of preemergence herbicides. Preemergence herbicides are heavily relied upon by growers to control weeds in major agronomic crops such as corn and soybean, but this tactic may be under threat in a changing climate. ARS researchers in Urbana, Illinois, in collaboration with university colleagues, addressed how variations in rainfall and soil temperature affect the activity of preemergence herbicides in corn. Data from previous studies was mined and analyzed with machine learning techniques to show that variable weather will impact the efficacy of preemergence herbicides. With increasing weather extremes and variations, integrated weed management is critical to the resiliency of agronomic cropping systems. (NP304, C3, PS3A, PS3B, Project No. 5012-12220-010-00D)

Gene editing in pink bollworm provides direct validation of gene function and resistance to transgenic cotton. Genetically engineered crops that produce insecticidal proteins from *Bacillus thuringiensis* (*Bt*) for managing insect pests are important globally, but the evolution of pest resistance to *Bt* crops reduces their benefit. Understanding the genetic basis of such resistance is needed to better monitor, manage, and counter pest resistance to *Bt* crops. ARS researchers in Maricopa, Arizona, and University of Arizona collaborators used CRISPR/Cas9 gene editing to introduce mutations in a key gene in a susceptible strain of pink bollworm, which resulted in resistance to Cry2Ab, a *Bt* toxin protein commercially used in transgenic cotton. Overall, 26 different disruptive mutations were found. These findings and previous results provide the first case of practical resistance to this toxin where evidence identifies a specific gene in which disruptive mutations can cause resistance and are associated with resistance in field-selected populations. This study confirms the genetic basis of resistance to this toxin in pink bollworm and shows that gene editing is useful for the direct functional validation of genes involved in pest resistance to *Bt* transgenic crops, critical information for preventing the loss of this powerful pest management strategy. (NP304, C3, PS3B, Project No. 2020-22620-023-00D)

Monitoring spread of apple maggot, a quarantine pest in the Pacific Northwest. Apple maggot flies are a quarantine pest of apples in certain regions of the U.S. Pacific Northwest. Identifying mechanisms apple maggot flies use for dispersal and colonization into new regions is important for maintaining apple maggot free zones. ARS researchers in Wapato, Washington, discovered that apple maggot larvae and pupae can survive in water for 2-12 days. These results indicate that immature stages of apple maggots can likely disperse in rivers, streams, and irrigation canals to establish in new habitats, such as apple maggot-free regions where apple is grown commercially. This information is being used by government agencies to monitor and prevent the spread of apple maggot flies into apple maggot-free zones. (NP304, C3, PS 3A, Project No. 2092-22430-003-00D)

Diets for mealworms and crickets composed of agricultural byproducts. Currently there is not a consistent, commercial source of feed specifically designed to grow crickets and mealworms. Mealworms are currently grown using wheat bran and some fresh vegetables, while crickets are mostly produced using modifications of chicken feeds. ARS researchers in Stoneville, Mississippi, developed mealworm and cricket diets that are composed of at least 80-percent agricultural byproducts, including rice bran (whole and defatted), spent brewery grain, corn distilled grain, canola meal, and brewer’s yeast. These new diets are specifically formulated to enhance growth and reproduction in mealworms and crickets, making them superior to the existing diets used by commercial producers. These diet formulations, if commercialized, could provide growers with a superior feed to raise mealworms and crickets for animal feed. (NP304, C3, PS3A, Project No. 6066-22000-092-00D)

An online operational tool for monitoring vector borne disease. European Biological Control Laboratory scientists in Greece contributed to the design and implementation of VectorMap-Gr, which is an online operational tool for entomological and epidemiological data monitoring to support timely and targeted vector control decisions. Using VectorMap-Gr, end users can obtain information on vector spatiotemporal dynamics; insecticide resistance status; occurrence of vector-borne diseases such as pathogens/infections in vectors, sentinel animals, and humans; and operationally relevant physical feature georeferenced data sets such as mosquito breeding sites. The tool, already implemented in Greece, may be readily adapted to other local vector-borne disease settings and vector surveillance/management programs. (NP304, C3, PS3c, Project No. 0212-22000-030-00D)

Predator bugs use multiple saliva and midgut enzymes to feed**.** Certain enzymes are important in the digestion, transportation, and processing of dietary lipids in insects. Understanding what enzymes are used when insects feed can lead to better artificial diets for mass production. ARS-associated scientists in Brisbane, Australia used gene analysis tools to identify different types of enzymes that break down lipids in the salivary gland and midgut of the predatory bug *Arma chinensis*. Additionally, the scientists discovered that the total activities of these enzymes were higher in the salivary gland than the midgut. The results suggested fatty materials can be added into the artificial diet for mass rearing of this insect, which preys upon a large variety of agricultural pests, including Colorado potato beetle, which has developed resistance to most insecticides. Developing an artificial diet to raise this insect will benefit agricultural producers who want to release it as a biological control agent. (NP304, C3, PS 3B, Project No. 0206-22000-003-00D)

Variable weather threatens future efficacy of preemergence herbicides. Growers often depend on preemergence herbicides to control weeds impacting major agronomic crops such as corn and soybean, but this tactic maybe under threat in a changing climate. ARS researchers in Urbana, Illinois, and University of Illinois Urbana-Champaign scientists mined and analyzed data from previous studies with machine learning to study how variations in rainfall and soil temperature affect the activity of preemergence herbicides in corn. Their results indicated that, with increasing extremes and variation in weather, integrated weed management is critical to the resiliency of agronomic cropping systems. (NP304, C3, PS3A, PS3B, Project No. 5012-12220-010-00D)

Biological control of the invasive cactus moth. ARS researchers in Tallahassee, Florida, have shown that commercial nopales and native prickly pear cactus species in the genus *Opuntia* are attacked by several insects, including the cactus moth (*Cactoblastis cactorum*). While the wasp species *Apanteles opuntiarum* is a good control agent against the moth, its success is related to geographical and ecological factors of release sites, as well as the ability to rear and release large numbers of these specialized parasites. Cooperative research with scientists in Mexico and ARS researchers in Tallahassee, Florida, showed that the release locations for biological control success are from the panhandle of Florida westward to Texas and into northern Mexico. Rearing studies demonstrated that wasps provided with cactus moth odors and pieces of host plant cactus enhanced parasitism rates in the laboratory. As a result, improved rearing methods for the wasps and release locations in Florida with good field characteristics have been included in a permit for release into the environment. Successful management of this invasive pest will now be accomplished along the Gulf Coast of the United States and into Mexico, potentially saving infestation and damage of more than 12,000 hectares of nopales agriculture in Mexico and large areas of native cactus in the United States. (NP304, C3, PS3B, Project No. 6036-22000-033-00D)

Identification of a gene controlling male European corn borer response to female pheromone. Farmers in the United States manage European corn borer (ECB) damage to maize by planting varieties that express one or more insecticidal *Bacillus thuringiensis* (*Bt*) proteins. *Bt*-resistant ECB populations have recently been detected in Canada, which is of great concern to the United States due to the borer's potential for spread. Two naturally occurring ECB strains with different pheromone (sex attractant) communication systems can inter-mate if located in the same geographic region, and the resulting genetic exchange between strains can affect how fast and how far *Bt* resistance spreads once it develops. An ARS researcher in Ames, Iowa, and a team of domestic and international collaborators discovered the gene that controls how ECB males specifically respond to different female pheromones—a significant accomplishment, because this is the first time this has been accomplished for any species of moth. This accomplishment opens the door for developing a genetic marker identifying the pheromone strain of ECB males collected in the field, which is crucial in estimating gene exchange rates between strains and the potential for the spread of *Bt* resistance among ECB populations. This information will be used by regulators, and scientists modeling development and spread of *Bt* resistance and potential ways to mitigate resistance problems. (NP304, C3, PS3B, Project No. 5030-22000-019-00D)

Development of a new method to study predation of a major cotton pest. Biological control of insect pests has immense economic value in many agricultural systems, but knowledge is limited about which predator species are most important for a given pest. ARS researchers in Maricopa, Arizona, developed a novel method for studying predation on the various life stages of *Lygus hesperus*, a major pest of cotton and other crops. The method tagged *Lygus* eggs, immatures, and adults with unique proteins that were subsequently detectable in predator guts. They found that big-eyed bugs and spiders were the numerically dominant predator taxa in the cotton field and the most frequent predators of immature *Lygus*. Results also showed that collops beetles and fire ants are adept at preying on the cryptic eggs, and that adult *Lygus*, sometimes engaged in cannibalism on immatures. The methods described will have value in the study of predator-prey dynamics in many agroecosystems. (NP304, C3, PS3B, Project No. 2020-22620-023-00D)

The value of biological control in the Asia-Pacific region. Biological control of insect pests has immense economic value in many agricultural systems throughout the world. Still, this value is underappreciated by many involved in spurring the innovation and adoption of biological control research and technology. An ARS researcher in Maricopa, Arizona, and partners in China, Australia, and the United Kingdom estimated that classical, introductory biological control against 43 insect pests in food, feed, and fiber crops in the Asia-Pacific region has an economic impact of $17.1-$22.7 billion USD annually. In addition, biological control was shown to promote rural growth and prosperity even in marginal, poorly endowed, non-rice environments. This research provides lessons for future efforts to mitigate invasive species, restore ecological resilience, and sustainably increase the output of global food systems. (NP304, C3, PS3B, Project No. 2020-22620-023-00D)

Mite control in pear orchards with the artificial sweetener erythritol. The artificial sweetener erythritol is non-toxic to humans but is insecticidal when ingested by certain insects and mites. Organic options are desperately needed for managing two key pests of pears, two spotted spider mite and pear rust mite, because existing products are not effective. However, producers do not want to use pesticides that harm their key predator, the western predatory mite. ARS researchers in Wapato, Washington, and Washington State University collaborators examined the effects of erythritol on pest and predatory mites. Results showed erythritol causes high mortality in rust and spider mites but does not kill western predatory mite. This demonstrates the potential of incorporating erythritol into mite management in pear orchards, without disrupting the natural control provided by predator mites. (NP304, C3, PS 3C, Project No. 2092-22430-003-00D)

A desert-adapted insect pest trap crop for the cotton agroecosystem. Cotton is vulnerable to a wide array of insect pests, but pest damage to cotton can be reduced by planting a more attractive host plant adjacent to cotton, also known as a companion trap crop. ARS researchers in Maricopa, Arizona, showed that vernonia, a desert-adapted plant, is strongly attractive to cotton pests and harbors an abundance of natural enemies. Furthermore, the scientists used a protein immunomarking technique to track the pests and captured very few arthropods beyond the vernonia trap crop. The arthropods’ strong attraction and fidelity to vernonia indicate that it could serve as a trap crop for the cotton pest complex and as a refuge for natural enemies. (NP304, C3, PS3B, Project No. 2020-22620-023-00D)

A highly diverse parasitoid complex that attacks psyllids. The potato psyllid vectors the pathogen that causes zebra chip, an economically important disease of potato worldwide. The establishment and expansion of potato psyllid populations on weedy host plants outside of potato crop fields can effectively be slowed by natural enemies of the potato psyllid. ARS researchers in Wapato, Washington, and Washington State University scientists collected specimens of psyllid species closely related to potato psyllid, and then examined the insects for the presence of Tamarixia parasitoids, which are known to parasitize potato psyllid in other regions. Results showed that psyllids associated with weedy chenopods, nettle, willows, field bindweed, and bitterbrush were parasitized by a previously unknown species of Tamarixia. These findings include some of the first parasite records for several of these psyllid species and suggest that a large and poorly known complex of psyllid parasitoids, which may include potato psyllid parasites, is common in habitats abutting U.S. Pacific Northwest potato fields. (NP304, C3, PS 3B, Project No. 2092-22000-022-00D)

Mating disruption for pear psylla investigated. Understanding factors that attract insect pests for mating opens avenues for developing new technologies for managing pests through mating disruption. Many psyllids are known to use acoustic cues in searching for mates, but this behavior had never been demonstrated for pear psylla, the most damaging insect pest of pears in North America. ARS researchers in Wapato, Washington, and Washington State University scientists developed methods to record, describe, and synthesize the acoustic signals of male and female pear psyllids that govern mate location behavior. Results showed that the two sexes engage in a formalized acoustic duet that allows searching males to locate singing females, and that males respond to a synthesized mimic of the female signal. These findings create an opportunity to develop a mating disruption program for pear psylla by hiding the true female signal by saturating pear orchards with the synthetic acoustic mimic. (NP304, C3, PS 3B, Project No. 2092-22430-003-00D)

Identification and characterization of SWD dsRNase. The current control for spotted-wing drosophila (SWD), a serious pest that can infest and destroy fruits of many plants, primarily relies on chemical insecticides. RNA interference (RNAi) is a safe and novel alternative control that is specific to SWD. Double stranded (ds) RNA may be applied in a field for SWD to ingest as a spray/bait to trigger RNAi, but its efficacy is limited due to dsRNA degradation in the fly's gut. ARS scientists in Corvallis, Oregon, identified two dsRNase genes from the SWD gut and investigated gene expression profiles during SWD life stages. dsRNases were produced in larval and adult stages during feeding periods. Research results are being used by scientists to improve RNAi application strategies for SWD control. (NP304, C3, PS3A, Project No. 0212-22000-030-00D)

New protein from corn inhibits pests. Insect and mold damage to corn causes millions of dollars in producer losses. Genes from corn that inhibit pests are a useful way of reducing damage. ARS scientists in Peoria, Illinois, isolated a gene from corn and put it into cultured corn cells. When insects fed on these corn cells, they grew more slowly and were up to 65 percent smaller than normal, and fungal growth on these cells was also reduced by as much as 70 percent. Using this gene in corn should reduce pest damage and result in improved yields and higher crop quality. (NP304, C3, PS3B and 3C, Project No. 5010-22410-023-00D)

Insecticide risk to monarch butterflies in Midwest United States. The eastern North American population of monarch butterflies has declined significantly over the last two decades, and assessments are needed to determine how insecticides affect the different life stages of monarch butterflies. ARS researchers in Ames, Iowa, and Iowa State University collaborators conducted dietary and contact toxicity studies on all monarch developmental stages using representative insecticides from four major classes. The diamide and pyrethroid classes were generally the most toxic insecticides to all life stages, and the neonicotinoid and organophosphate were generally the least toxic. Toxicity results were compared to insecticide exposure estimates derived from a spray drift model and from previous data on insecticide levels detected on or in milkweed tissue. Aerial applications of foliar insecticides could cause high mortality in larvae and eggs located near the field and downwind, with lower mortality predicted for adults and pupae. However, given the predicted levels of exposure and the highly mobile behavior of monarch females that lay their eggs in many locations throughout a landscape, these results suggest that benefits of establishing new habitat close to maize and soybean fields in agricultural landscapes outweigh negative effects of insecticides on monarch population growth in the north central United States. This information will be used by regulators and scientists planning monarch habitat restoration. (NP304, C3, PS3B, Project No. 5030-22000-019-00D)

Monarch larvae are not affected by double-stranded RNA used to control Varroa mites in beehives. Varroa mites are parasites that contribute to high levels of honeybee colony losses. A varroa-active double-stranded RNA (dsRNA) was recently developed to control varroa mites by RNAi gene silencing. This dsRNA kills varroa mites by binding to a region in the mite's calmodulin gene (cam), thus disabling the gene. This gene is similar to the cam gene in monarch butterflies. ARS researchers in Ames, Iowa, and Iowa State University collaborators exposed monarch larva to one- and ten-fold higher concentrations of varroa-active dsRNA than are used to treat honeybee hives. The varroa mite and monarch-active dsRNA did not significantly affect larval mortality, larval or pupal development, pupal weights, or rates of adult emergence from the pupa. The results indicate that dsRNA, including the varroa-active dsRNA eaten by monarch larvae, are deactivated before they can interact with the target gene, and do not pose a hazard to monarch larvae. This information will be useful to monarch conservation groups, beekeepers, and the honeybee industry, public-sector, government scientists, and regulatory agencies. (NP304, C3, PS3B, Project No. 5030-22000-019-00D)

A genetic assay for detecting an invasive beetle from wood frass. Obscure species such as wood boring or longhorned beetles live most of their immature lives in wood, so it is difficult to identify larvae of species. This hinders controls measures and enables the beetles to quickly invade a new environment, so methods are needed to identify beetle species at the larval stage or from non-destructive sampling methods. The Queensland longhorned beetle, an invasive species on Hawaii island with a broad host range, is spreading quickly and is difficult to detect. To rapidly identify host species in the absence of larval tissue, ARS scientists in Hilo, Hawaii, developed a genetic assay to detect the presence of beetle DNA from frass material. This assay is currently being used to identify new hosts and the range of this species in the Hawaiian Islands. (NP304, C3, PS 3A; Project No.: 2040-22430-027-00D)

A parasitoid used to control pests transmits citrus greening. Parasitoids are frequently used in biological control and integrated pest management strategies all over the world. ARS researchers in Fort Pierce, Florida, demonstrated for the first time a risk posed by these control agents. Deployment of parasitoids has been promoted as a means of suppressing Asian citrus psyllid (ACP), a pest that transmits citrus greening disease, or Huanglongbing, in citrus orchards. However, while controlling ACP, the parasitoid *Tamarixia radiata* can inadvertently vector the Huanglongbing pathogen known as CLas. This diminishes *Tamarixia radiata*'s biological control efficiency, particularly in orchards where CLas-infected and uninfected asymptomatic citrus trees coexist. This finding presents a new and significant caution to the strategy of implementing biological control using parasitic wasps. (NP304, C3, PS3B and 3C, Project No. 6034-22320-007-00D)

The field bindweed psyllid is not a direct threat to potato. Zebra chip disease of potato is caused by a pathogen called Liberibacter that is acquired by potato psyllids when they feed on the phloem of potato plants. A close relative of potato psyllid, the field bindweed psyllid, also carries Liberibacter, but it was never clear whether the psyllid transmits the pathogen to potato. ARS researchers in Wapato, Washington, collaborated with Washington State University and University of Idaho scientists and compared feeding behaviors of bindweed psyllid on host plant (field bindweed) tissues and the non-host potato. Researchers found that the psyllid readily feeds on the phloem of field bindweed but avoids the phloem of potato. Because bindweed psyllid avoids the phloem of potato, it is believed that this species is unlikely to transmit Liberibacter to potato. These results contribute to information about how zebra chip disease is spread and provides important behavioral and biological insight into host plant use by psyllids. (NP304, C3, PS 3B, 2092-22000-022-00D)

Developing a conservation biological control strategy for Asian citrus psyllid. Growers rely on insecticides to control Asian citrus psyllid, the vector of citrus greening disease, but insecticide-resistant psyllid populations are emerging, and control costs are high. ARS scientists in Fort Pierce, Florida, are developing a control strategy called Conservation Biological Control in which certain plants are grown to support the insect predators that attack the psyllid. The scientists demonstrated that a statistical method called Response Surface Methodology (RSM) could be used to optimize mixtures of plants to support the insect predators of the psyllid. RSM analysis showed that predator occurrence was influenced by individual plant species as well as the aggregate of species. Predator abundance was highest in the spring and decreased in warmer summer months. The results showed that the plant species mix—both type and numbers—drives predator occurrence. This suggests that the proportion of each plant species included in the mixture should be considered when formulating a plant mixture to support biological control of psyllid. (NP304, C3, PS3B and 3C, Project No. 6034-22320-007-00D)

Modeling grasshopper outbreaks in response to El Niño. Grasshopper outbreaks in the United States annually destroy approximately 20 percent of rangeland forage valued at approximately $1.67 billion and are major threats to crops throughout the western United States. Periodic droughts associated with long-term climate cycles, like El Niño, may be one factor causing grasshopper outbreaks. ARS researchers in Sidney, Montana, compared grasshopper outbreaks reported over the last 70 years to long-term climate records. Grasshopper modeling revealed that grasshopper densities in the Northern Great Plains decreased during peak El Niño years, but grasshoppers in the Southern Great Plains increased to outbreak levels during these time periods. Modeling results will help prioritize grasshopper monitoring and treatments in advance of outbreaks to maximize management effectiveness. (NP304, C3, PS3A, PS3B, Project No. 3032-22000-019-00D)

First discovery of an introduced biological control agent on Bagrada bug in California. Bagrada bug is a major recent (since 2008) non-native pest of cole crops like broccoli, cauliflower, and cabbage. In California, the national leader in production, these crops are worth more than $2.3B per year. Control is difficult because bagrada bug, unlike most plant bugs, lays its eggs in the soil. ARS researchers in Albany, California, discovered a tiny parasitic wasp, *Gryon aetherium*, in north-central California in 2020 and identified it in 2021. The wasp had emerged from bagrada bug eggs collected from the soil under infested host plants. Laboratory breeding experiments confirmed that it is the same species as a candidate biological control agent being tested in quarantine, although a genetically different strain. This is the first discovery that this parasitic wasp is already present in California. Its ability to attack bagrada bug eggs in soil is currently being verified. If found to be safe and effective, future rearing and release of large numbers of this parasite could significantly reduce economic losses caused by bagrada bug. (NP304, C3, PS2B, Project No. 2030-22000-031-00D)

Discovery of safer pesticides. For the past 20 years, botanic pesticides have received acclaim and recognition as attractive alternatives to synthetic pesticides for pest management due to their reduced threat to human and environmental health. In 2018, ARS scientists in Beltsville, Maryland, showed that methyl benzoate (MB), a volatile organic compound (VOC) from fermented apple juice, exhibited significant insecticidal activity against invasive spotted wing drosophila (SWD). The scientists showed that some MB analogs can kill or repel many insects and non-insect pests in various stages of development, including mosquitoes, bed bugs, fire ants, ticks, flies, moths, and nematodes, through contact or fumigation. These MB analogs will provide growers with an environmentally friendly alternative to synthetic pesticides for managing insects and non-insect pests and have great potential to be used as safe pesticides for human protection. (NP304, C3, PS3B, Project No. 8042-22000-315-00D)

Virus transmission unveiled. Not all whiteflies pose equal threats as vectors of plant viruses. Since first described more than 100 years ago, the whitefly *Bemisia tabaci* has become an agricultural pest distributed worldwide. It causes direct cosmetic damage to various crops during feeding and by the growth of sooty mold fungus in its sugar-rich honeydew secretions on plants. However, the most severe damage caused by the whitefly is its ability to spread plant viruses. *B. tabaci* is a vector for more than 100 different plant viruses, primarily the plant-infecting begomoviruses. ARS scientists in Ithaca, New York, and collaborators at the University of Washington and the Volcani Center in Israel discovered that nine different *B. tabaci* populations collected in Croatia and Israel vary in their ability to transmit or spread the begomovirus tomato yellow leaf curl virus. Some populations spread the virus very efficiently, while other populations did not spread the virus readily between plants. Differences in *B. tabaci* proteins involved in virus transmission were found among these populations. Understanding the proteins that regulate virus transmission will lead to the development of novel strategies that block virus spread within a crop. (NP304, C3, PS3a, 3b, and 3c, Project No. 8062-22410-007-00D)

Modeling potential U.S. invasion by the Central American locust. The Central American locust is a large, swarming locust that is endemic to Central America. Swarms of this locust can devour crops like rice, wheat, citrus, and lentil over very large geographic areas and inflict millions of dollars in economic harm. There is concern that changing climate conditions may enable the Central American locust to invade the United States in the future. ARS researchers in Sidney, Montana, developed statistical models to assess the chance of invasion by Central American locusts. Modeling results suggest that locations in Central Texas and Southern Arizona may be vulnerable to locust invasion beginning around the year 2040, but environmental conditions may not contribute to outbreaks until the year 2060. (NP304, C3, PS3A, PS3B, Project No. 3032-22000-019-00D)

Mormon cricket growth rate increases with elevation. Many managers use degree day estimates of pest occurrence after spring thawing to plan surveying and control efforts. ARS researchers in Sidney, Montana, investigated the growth rate of Mormon crickets across elevations from 300 to 9000 feet. The minimum temperature for growth declined from 61°F to 58°F with increasing elevation and the growing degree days required to reach adult remained the same among populations. In general, a base temperature of 60°F for mid-elevation and 916-degree days accumulated from egg hatching to adult are reasonable estimates for making insecticide applications from sea level to 9000 feet. (NP304, C3, PS3A, PS3B, Project No. 3032-22000-019-00D)

Pest complexes associated with novel bioenergy and cover crops in the Northern Plains. Pests of novel crops can become reservoirs for traditional cash crops in a region. ARS researchers in Sidney, Montana, surveyed insect pest communities in two bioenergy crops, carinata or camelina, and a 10-species forage/cover crop. Crucifer flea beetles were the dominant pests associated with carinata and the cover crop mix while the pest complex associated with camelina was dominated by generalist lygus bugs. Crucifer flea beetle have the potential to become serious pests of carinata. Camelina is less likely to be affected or serve as a reservoir and could be a good fit to replace fallow and rotate with canola. (NP304, C3, PS3B, PS3C, Project No. 3032-22000-019-00D)

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

There are more insect pests in citrus orchards in China than previously known**.** Citrus is an important commercial crop throughout the world where it is grown, and citrus pests severely limit production and economic returns. Scientists in the ARS-associated laboratory in Beijing, China, conducted surveys of citrus pests in China, which is considered to be one of the centers of origin of citrus. Collected insects from citrus orchards in China underwent DNA analysis and were grouped as either pests or beneficials. Results showed that the number of pests is much higher than has been documented in literature. The data generated in this study provide a valuable resource for research in a broad range of areas, such as citrus pest management and monitoring programs. (NP304, C4, PS 4A, Project No. 0206-22000-003-00D)

Methyl bromide alternatives to control invasive and quarantine horticultural insect pests. Methyl bromide (MB) is a broad-spectrum fumigant used largely in several countries for combating soil-borne pests and others in postharvest, pre-shipment, and quarantine treatments. Despite its biological effectiveness, the fumigant has been identified as a major ozone depleting substance, and its use needs to be phased out in countries where it is still being used. ARS researchers in Parlier, California, conducted studies to optimize, develop, and register MB alternatives to support regulatory compliance and enhance global food security. The researchers developed a novel postharvest phosphine fumigation to control the devastating spotted wing drosophila in fresh citrus exports from California to New Zealand, which are valued at $12 million annually. In addition, a novel postharvest sulfuryl fluoride fumigation was developed to control other economically important insect pests, including navel orangeworm, almond moth, and Mediterranean flour moth, in almond exports from California to India, which are valued at $2 billion annually. The research directly resulted in market retention or expansion and served as the basis for technical interaction between industry, USDA Foreign Agricultural Service, USDA Animal and Plant Health Inspection Service, U.S. Environmental Protection Agency, and respective counterparts in foreign governments. (NP304, C4, PS 4A, Project No. 2034-43000-042-00D)

Effects of nitric oxide fumigation in controlling microbes on unshelled peanuts. Stored products such as peanuts are vulnerable to pest infestation and microbial infection. Methyl bromide is a broad-spectrum fumigant commonly used to control crop pests and pathogens in postharvest, pre-shipment, and quarantine treatments. Despite its biological effectiveness, the fumigant has been identified as one of the major ozone depleting substance and is being phased out worldwide, including the United States. ARS researchers in Salinas, California, conducted research to expand the use of nitric oxide, a newly discovered fumigant, to control both pests and microbes. Researchers fumigated unshelled peanuts with different rates of nitric oxide at different temperatures and demonstrated that nitric oxide was able to achieve 100 percent reduction of microbe loads on the treated peanuts. The results suggest that nitric oxide fumigation has the potential to control microbes on unshelled peanuts and will greatly benefit peanut producers and processors. (NP304, C4, PS 4A; Project No. 2038-22430-003-00D)

Efficacy of nitric oxide fumigation against light brown apple moth. Light brown apple moth has been established in California since it was first detected more than 10 years ago. Effective quarantine treatments are needed because this pest has a very broad host range, very limited distribution, and is quarantined in most countries. ARS researchers in Salinas, California, determined the effectiveness of different concentrations of nitric oxide against different life stages of this insect pest. Complete control of the moth larvae and pupae was achieved in 8 to 24 hours. These results indicate that nitric oxide can be suitable for postharvest control of light brown apple moth and, therefore, has the potential to facilitate export of fresh fruits and vegetables. (NP304, C4, PS 4A; Project No. 2038-22430-003-00D)

Efficacy of essential oil anisole as a potential fumigant for postharvest pest control. There is a need for environmentally friendly fumigants for postharvest pest control. The plant essential oil anisole is insecticidal and has potential as an alternative fumigant because it has a smaller molecular size and higher vapor pressure than most other plant essential oils. ARS researchers in Salinas, California, evaluated anisole as a potential fumigant for postharvest pest control and tested anisole against several insect pests. Anisole fumigation was effective against adults of three stored product insects, including rice weevil, granary weevil, and confused flour beetle, as well as against larvae and adults of western flower thrips. Complete control of all four insect pests was achieved in as a little as 4 to 24 hours, depending on anisole dose and insect species. These research results indicate that anisole has potential as a fumigant for postharvest pest control, which will greatly benefit the fresh and stored product industries. (NP304, C4, PS 4A, Project No. 2038-22430-003-00D)

<u>Expanding mating disruption for controlling stored product insects.</u> Mating disruption requires large amounts of sex pheromones to confuse insects and interfere with mating. This approach has not been tested in certain postharvest settings, such as in stored products. ARS researchers in Manhattan, Kansas, evaluated the effectiveness of different dispenser densities on Indianmeal moth in retail pet stores and showed that the treatment could be effective, although it took time for populations to decline to a low and stable level. This research is critical for improving and expanding this pest management approach. (NP304, C4, PS4B, Project No. 3020-43000-033-00D)

<u>Netting with incorporated insecticide as a new management tactic for postharvest pests.</u> Insects can enter food facilities through vents, gaps around doors, windows, eaves, and other openings, causing major economic losses to high value commodities. ARS researchers in Manhattan, Kansas, demonstrated the effectiveness of netting containing the insecticide deltamethrin to disrupt movement of several different species of stored product insects. The study strongly suggests that netting and packaging materials treated with insecticide can be used in multiple integrated pest management strategies to protect commodities across the entire postharvest agricultural supply chain. (NP304, C4, PS4B, Project No. 3020-43000-033-00D)

<u>Quantifying risks of insect infestations of gluten-free and low-gluten grain and flour products.</u> Insects infesting stored products cause significant damage to wheat and wheat-based products. Alternatives to traditional wheat flour have increased in popularity and the market for these products is now valued at more than $21 billion. However, little is known about the ability of stored insects to infest and survive on these commodities. ARS researchers in Manhattan, Kansas, assessed the ability of insects infesting stored products to exploit sorghum grain and develop on flour derived from sorghum and other grains. They found red flour beetles could develop on oat, rice, rye, buckwheat, barley, spelt, teff, and sorghum flours, but that they developed more slowly on sorghum and quinoa flours. As use of alternative flours increases, a better understanding of potential risk of insect infestations will be important for facility managers. (NP304, C4, PS4B, Project No. 3020-43000-033-00D)

<u>Improving the efficacy of phosphine fumigations for stored product insects.</u> Fumigation with phosphine is one method to help control postharvest insect infestations in stored products, but resistance to phosphine is becoming common among a variety of stored product insects. ARS

researchers in Manhattan, Kansas, tested a new inexpensive dosimeter system and compared its accuracy to more expensive electronic fumigation monitoring systems. A new marker was discovered that can be quickly and accurately used to identify populations of red flour beetle that have strong resistance to phosphine. New methods improve monitoring of phosphine fumigations and their potential efficacy. (NP304, C4, PS4B, Project No. 3020-43000-033-00D)

Expanding genomics resources for stored product insects. More than 50 different species of stored product insects are recognized as pests worldwide. Genome sequences can be used to better understand the complex biology of these insects and develop new tactics to control populations. Through a partnership with the Ag100Pest initiative, ARS researchers in Manhattan, Kansas, and other ARS locations implemented new long-read sequencing technologies to generate chromosome scale assemblies of 20 prominent global postharvest pests. This information will be used to determine whether common features of chemosensory genes allow this broad taxonomic range of insects to respond to the same food odors and will ultimately lead to the development and optimization of new management tactics. (NP304, C4, PS4B, Project No. 3020-43000-033-00D)

Optimizing aerosol insecticides for improved stored product insect management. Applying insecticides as an aerosol to improve coverage inside food facilities is a widely used tactic of integrated pest management in the food industry. However, there has been limited information available on the effectiveness of these treatments. ARS researchers in Manhattan, Kansas, showed that droplet size distributions and droplet concentration varied considerably within a flour mill, which resulted in spatial variation in efficacy against the confused flour beetle. Compared to handheld sprayers, compressed gas sprayers produced more uniform droplet sizes and had higher spray coverage areas. This study will help improve efficacy of aerosol insecticide applications for management of stored product insects. (NP304, C4, PS4B, Project No. 3020-43000-033-00D)