United States Department of Agriculture
Agricultural Research Service

National Program 304 • Crop Protection and Quarantine

FY 2022 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 2022. 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 2022 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

Exact distribution of varieties and genotypes of a noxious weed. Common crupina is a noxious weed in the western United States that forms solid stands and reduces the quantity and quality of forage. ARS scientists in Sidney, Montana, conducted molecular analyses on crupina samples from its native range in southern Europe and compared them with samples from Idaho, Washington, and Montana to help determine the geographic origins of its invasion. Seven distinct genotypes were identified. A fungal biological control agent that significantly reduces crupina growth and seed production is proposed for release by ARS in 2022. The molecular research will allow monitoring of efficacy of the fungus on all genotypes of crupina in distinct habitats, ultimately increasing efficiency of the biological control agent.

Tracing the origins of Roseau cane scale. ARS scientists and collaborators identified the origins of the non-native Roseau cane scale (*Nipponaclerda biwakoensis*), an insect pest damaging common reed in the Mississippi River Delta (MRD). The scale has contributed toward extensive die-off of common reeds in Louisiana, which protect the marsh ecosystem from erosion and storm-related impacts, stabilize shipping channels, and shield critical infrastructure. ARS scientists in Beltsville, Maryland, in collaboration with APHIS, led a multinational effort to identify the origins of the introduced population. Genetic data from East Asian and U.S. populations pinpointed northeastern China as the likely source. Ecological niche modeling was used to compare the pest's native range to MRD, matching for climatic similarity. The combined results provide critical guidance to focus future biological control efforts, narrowing the search for natural enemies that may be successfully introduced to control the scale in Louisiana. This research contributes toward the protection and restoration of a critical natural resource and gateway to a major shipping hub in the United States.

New biological attributes and host records discovered for wasps important to agriculture and natural resources. Correct identification of biological control agents, as well as an understanding of their biological role in mitigating pest species populations, is essential for making rearing and quarantine decisions in the protection of U.S. agricultural interests worldwide. ARS scientists in Beltsville, Maryland, in a joint project with other scientists in Canada, France, Germany, Mexico, Florida, North Carolina, Pennsylvania, and Washington, D.C., produced the first complete phylogenomic analysis of Hymenoptera (wasp) relationships, as well as a more thorough analyses within superfamilies. This Big Data project includes more than 4,000 species of Hymenoptera, and 210,000 base pairs of data, requiring super computers for analysis.

Genome of lesser grain borer provides new insights into insecticide resistance and habitat usage. The lesser grain borer is a major pest of grain worldwide, and phosphine-resistant borer populations threaten the effectiveness of fumigations for pest control, which is a key concern for stakeholders. ARS researchers in Manhattan, Kansas, sequenced the genome of this grain borer to understand its biology and habitat usage. Compared to other beetle species, this species lacks a significant expansion of genes related to resistance to contact insecticides. The immature larval stage feeds inside grain kernels, reducing its exposure to insecticides and potentially reducing the pressure to evolve detoxification mechanisms against these chemicals. In addition, even though this species was reported to feed on wood and trees, its genome does not code for any enzymes that can degrade plant cell walls. Therefore, it likely cannot survive while feeding on wood, but rather, it may use wood and trees found in the field for refuge and shelter in agricultural landscapes. Overall, this genome provides a useful tool for studying phosphine and insecticide resistance in stored product insects and clarifies some misnomers regarding the biology of lesser grain borer.

Component 2 – Weeds

Quarantine evaluation of a biological control agent for earleaf Acacia. Biological control agents are urgently required for earleaf Acacia (*Acacia auriculiformis)*, an Australian native tree invasive in Florida. Without control this invasive tree could rapidly expand, reducing biodiversity by invading protected areas such as the Everglades National Park. Scientists at the ARS-associated laboratory in Brisbane, Australia, conducted foreign exploration trips to search for potential biocontrol agents and determined that a gall-forming wasp should be prioritized for evaluation as a biological control agent, as a close relative of this wasp has successfully controlled Acacia spp. in Africa. The scientists completed preliminary host range testing and impact assessments in Brisbane and found that this wasp specifically targets and damages earleaf Acacia seedlings. This potential biological control agent was sent to ARS quarantine facilities in the United States for final testing and to assess if it might harm non-target organisms in Florida ecosystems.

BenchBot: A low-cost autonomous robot for high-throughput phenotyping and building image repositories. High-throughput phenotyping systems for greenhouses and semi-field conditions are critical for plant breeding and building image repositories that use computer vision and artificial intelligence for mapping cash crops, cover crops, and weeds. However, these high-throughput phenotyping systems are costly, resulting in limited use. ARS scientists in Beltsville, Maryland, designed and built BenchBot, a fully autonomous robotic platform, in collaboration with North Carolina State University researchers. BenchBot costs less than $20,000, significantly less than most commercial and research grade systems that cost millions of dollars. Designs for BenchBot are published on GitHub and are being used for high-throughput phenotyping by ARS and university scientists at North Carolina State University and Texas A&M for building a national agronomic plant image repository. BenchBot is a low-cost, user-friendly technology that is making high throughput phenotyping accessible for researchers.

Reproductive variation in Russian knapweed may influence biocontrol efficacy. To manage invasive plant species effectively, information is needed about how they reproduce. ARS researchers in Sidney, Montana, used genetic markers to determine how recruitment of invasive Russian knapweed (*Acroptilon repens*) varies across its range. This species forms patches and can spread by both root growth and seed. No shared genotypes were found between 41 western North American populations, indicating that Russian knapweed is spreading via seed to distant locations. A correlation was found between latitude and clonal (spreading via roots) vs. seed reproduction; clonality was observed more often in northern latitudes. This trend was associated with decreasing maximum annual temperature and 30-year average of available growing degree-days and increasing soil organic carbon content. These results have management implications: if not properly implemented, grazing or herbicide applications that create open spaces for recruitment may increase the likelihood of Russian knapweed patch persistence through seed, and recently released galling biological control agents in North America may be less effective in northern latitudes where Russian knapweed spread by seed is less prevalent.

Mammals play an understudied role in Russian olive seed dispersal. Russian olive produces large berries that should attract seed consumption by vertebrates, but few data are available on the potential for seed dispersal by animals. ARS scientists in Sidney, Montana, gathered coyote and porcupine fecal material from within Russian olive windbreaks, and used numerous intact Russian olive seeds that were found within the scat to perform germination tests. Overall, seeds that passed through coyote and porcupine intestinal tracts germinated equally or higher than control seeds (not passed through an intestinal tract). These data provide some of the only empirical evidence that viable Russian olive seed can be spread via endozoochory (seed movement by animals) from mammals. This is also the first documented occurrence of endozoochory of any plant species by porcupines. Due to this novel finding, this research was recently highlighted by The Wildlife Society.

Developing strategies to support invasive annual grass management. Invasive annual grasses such as bromes (*Bromus* spp.), ventenata (*Ventenata dubia*) and medusahead (*Taeniatherum caput-medusae*) are problematic throughout arid and semi-arid rangeland ecosystems of the western United States, with substantial impacts to ecosystem health and forage productivity. ARS researchers in Sidney, Montana, and collaborators are developing effective control strategies that reduce wildfire spread and are documenting invasive annual grass responses to fire in the Great Plains. Researchers employed U.S. Forest Service fire spread models in a first-of-its-kind exercise to determine which plant traits contribute to effective greenstrips (linear strips of less-flammable species planted to interrupt wildfire spread) that provide fire protection and staging areas for firefighters to safely initiate control operations. Field data and remotely sensed products were also combined to verify that rangeland fire management in the eastern Great Plains is compatible with strategies to limit the frequency and abundance of invasive annual grasses. These “lessons learned” from the Great Plains were included in national efforts to describe the total ARS impact on knowledge of invasive annual grass management.

Climate change necessitates near-absolute weed control in corn and soybean. Estimates of future grain yields in the face of climate change assume weed-free conditions, but given the adaptability of weeds (e.g., the current epidemic in herbicide resistance), this assumption may not be realistic. ARS researchers in Urbana, Illinois, and university colleagues used a novel approach with existing herbicide evaluation trials conducted throughout 3 decades to identify the main drivers of yield loss in corn and soybean. Abnormally hot or dry conditions that were found during flowering—conditions expected to occur more frequently in much of the U.S. Corn Belt—exacerbated crop losses when weed control was less than absolute. As agriculture adapts to climate change, this research underscores the critical importance of developing more effective integrated weed management systems to help producers maximize yields and feed a growing population.

Bluebunch wheatgrass and Sandberg's bluegrass can be harvested and stored. Restoring invaded and degraded rangelands is central to recovering the health and function of rangeland throughout the western United States. Federal land managers and livestock producers in this region have found restoration of these systems to be very difficult because native plants rarely establish from seeds. ARS scientists in Burns, Oregon, are working on a novel restoration system that includes using buds collected from native plant crowns and stored for planned restoration efforts. Their findings suggest that buds of bluebunch wheatgrass and Sandberg's bluegrass can be harvested mechanically and stored at about 40^{o} F. Bud longevity is associated with the amount of material that supports and surrounds the group of buds: The greater the amount of supporting material, the longer the stored bud remains viable. This is critically important to developing the new restoration system because crowns must be easily collectible and storable to be useful to managers.

<u>Seeds increase leafy spurge abundance.</u> Understanding if weeds are reproducing primarily through clones or seedling recruitment is particularly important for sustained management with biological control (biocontrol), as it relies on specialized insects with selective feeding patterns to limit weed populations. ARS researchers in Sidney, Montana, surveyed weed density, genetic diversity, and associated biocontrol agent abundance (Aphthona species flea beetles) in 100 leafy spurge populations across North Dakota, Montana, and Idaho. This survey provided insight into 1) how frequently seedlings contribute to population growth, and thus the density of leafy spurge infestations; and 2) whether leafy spurge density is related to the abundance of biocontrol agents and the balance between clonal and seedling recruitment into local populations. No evidence was found of frequent recruitment from seed at all sites. Aphthona spp. flea beetles were everywhere, but the association between the number of flea beetles and leafy spurge density was not consistent. Increased importance of seed production may be changing the most effective targets for biocontrol after decades of Aphthona pressure on leafy spurge invasions. Further testing is needed to ensure biocontrol management is sufficient for long-term sustainable control.

<u>Determining the mode of action of spliceostatin C.</u> Spliceostatin C (spC), one of the bioactive components produced by the soil bacterium *Burkholderia rinojensis*, displayed a high phytotoxic activity at low doses against several weeds. However, the precise mechanism of action of spC is yet to be elucidated. ARS researchers in Oxford, Mississippi, analyzed the genomic sequences for 13 spC susceptible and resistant weeds and found genetic mutations that could explain the range of toxic effects of spC on these weeds. These findings provide important information for further investigation into the possibility of generating spC-resistant crops through genetic engineering and breeding and developing spC as a bioherbicide for weed management targeting herbicide-resistant weeds.

<u>Modern satellites improve the range of cover crop biomass estimates.</u> Cover crops are the premier climate smart farming practice, but their benefits vary due to the spatial variability of soil fertility and drainage within a given field. Technologies that map cover crops will facilitate variable-rate technologies that apply inputs based on cover crop performance within a field, thus enabling the merging of precision and sustainable agriculture. ARS and USGS scientists in Beltsville, Maryland, conducted a 3-year observational study assessing the ability of two modern satellites to predict cover crop biomass at 20-meter spatial resolution. A modern spectral index using the "red-edge" spectral region improved prediction of cover crop biomass from 1,500 kg ha-1 to 1,900 kg ha-1. This accomplishment is an important step toward

increasing abilities to estimate cover crop biomass and will aid in mapping cover crops to facilitate precision application of inputs (e.g., herbicides, fertilizer) that correspond with their spatial performance in a field.

Satellites can be used to estimate timing of cover crop termination. Cost-share programs in Maryland and Delaware distribute incentive payments for planting cover crops and verifying producer participation depends in part on physical visits by state or country agents to confirm cover crops termination has occurred. To reduce agent workloads, ARS and USGS scientists in Beltsville, Maryland, downloaded, processed, and extracted a timeseries of the normalized difference vegetation index for each enrolled field. Using a within-season termination algorithm developed by the ARS and USGS scientists, about 84 percent of reported cover crop termination dates can be predicted within a 1.5-week margin of error before and after termination. Accurately estimating cover crop termination date is increasingly important since Chesapeake Bay states began increasing incentives for later termination of cover crops (after May 1). Satellite imagery can provide a fast and consistent approach for generating cover crop termination maps federal and private cost-share programs can use to verify management.

Pollinators associated with Russian olive. Russian olive is spreading and limiting native vegetation within riparian zones and a biological control program that targets flowers and seed production is under development to limit its spread. ARS researchers in Sidney, Montana, used an insect exclusion experiment to determine whether Russian olive depends on insects for pollen transport and subsequent seed production. Additionally, surveys were conducted for 2 years to track insects that visited flowers. The scientist found numerous insect species visited Russian olive flowers, including 13 polylectic native bee species, but most of the visitors were western honey bees, which comprised more than 80 percent of all insect visits. Nearly 35 percent of flowers that were allowed potential insect visitation produced fruit/seeds, compared to 5 percent of flowers in which insect visitation was excluded. These findings indicate that numerous insects utilize Russian olive flowers, and that insect visitation is needed for high fruit/seed output. However, honey bees were the most frequent floral visitors, and Russian olive flowers may provide a less frequent resource for native pollinators.

Component 3 – Insects and Mites

Plant symbiont systems deliver crop protectants. A major challenge in plant disease control is delivering protectants to the plant vascular system to control pathogens and the insects that transmit them via feeding on plant vessels. A plant symbiont-based system (Symbiont™) developed by ARS researchers promises to be a cost-effective method for delivering biological therapeutic molecules that control citrus greening disease (Huanglongbing, or HLB) devastating the citrus industry in Florida. Traditionally, scientists use *Agrobacterium*, a common bacterium, to modify plant genes to generate transgenic plants that enable farmers to protect their crops against harmful insect pests and pathogens. However, transgenic plant adoption in agriculture has been limited, largely due to concerns over potential environmental impacts and the cost and time associated with environmental impact studies needed for regulatory approval of transgenic crops. ARS researchers in Fort Pierce, Florida, and Ithaca, New York, worked with a small Florida agribusiness to develop a method that, for the first time, used *Agrobacterium* to engineer independently growing plant cells, referred to as 'Symbionts', to produce molecules that can modify plant traits. When transplanted onto a plant, these engineered symbionts provide real-time delivery of desirable plant traits, eliminating the need to make the plant transgenic. Their results demonstrated that symbionts on citrus trees lasted more than 2 years without producing harmful effects to the trees, and improved symbiont transplantation and inoculation methods to produce more uniform and rapid growing symbionts. The results show that the Symbiont system has potential to efficiently deliver therapeutic molecules to the difficult-to-reach vascular system where it is needed most for controlling vectored plant diseases.

<u>Pesticides registrations for food, nursery, and floral crops.</u> The IR-4 program supports research and testing to ensure that new and more effective crop protection products are developed and available for minor and specialty crop producers. These efforts require effective collaborations among federal agencies, the crop protection industry, and land-grant colleges and universities. ARS researchers in Charleston, South Carolina, coordinated ARS research at their location and in Corvallis, Oregon; Salinas, California; Tifton, Georgia; Wapato, Washington; and Wooster, Ohio, to conduct IR-4 Project research advancing the registration of pesticides for specialty crop growers (fruits, vegetables, nuts, flowers, nursery and landscape plants, and Christmas trees). ARS research support resulted in 17 new tolerances and 96 food crop registrations and affected 226 decisions on non-food ornamental crops. Michigan State University's Center for Economic Analysis recently published that IR-4 Project efforts adds $8.7 billion dollars to the annual gross domestic product. Based on these values and on calculations that ARS efforts make up 15 percent of the efforts, ARS contributions to the IR-4 Project result in $1.3 billion dollars in annual impact.

<u>Fourteen-year study documents significant lady beetle declines in field crops.</u> As major predators of aphids and other pests, lady beetles provide important agroecosystem services to field crops. However, several native lady beetle species in North America have declined because of invasive lady beetles. Research showed that populations of three different native species declined drastically or essentially disappeared from agricultural landscapes in eastern South Dakota following establishment of an invasive ladybeetle species in the 1980s. ARS scientists in Brookings, South Dakota, surveyed five types of field crops and restored prairie in eastern South Dakota from 2007 to 2020 and found significant declining trends were evident in both corn and soybean. Declines of lady beetles in these crops in late summer could substantially reduce overwintering populations that emerge to colonize early-season spring crops, thus potentially disrupting biocontrol services in cropland both early and late in the season. These findings are of concern because reduced pest control benefits provided by lady beetles may lead to increased use of insecticides in agricultural fields, increasing crop production costs and environmental impacts.

<u>Rootworm resistance to *Bacillus thuringiensis* linked to altered bacterial community</u>. Rapid development of resistance is threatening one of the few remaining management options that

U.S. Corn Belt growers have for controlling corn rootworm, a pest that varies in its levels of resistance and causes $2 billion in losses from yield loss and management costs. ARS scientists in Columbia, Missouri, and University of Missouri researchers evaluated corn rootworm resistance to maize that expresses rootworm-specific toxins in the field. The scientists found that resistant larvae harbored fewer bacteria and that the bacteria were different than the bacteria found in susceptible larvae; in addition, susceptible larvae that fed on toxin-expressing maize subsequently had major changes in bacterial populations, but the bacteria in resistant insects remained relatively unchanged. These results demonstrate that toxin resistance in corn rootworm is associated with bacterial alterations and suggests that corn rootworm microbes could be manipulated by seed coatings, soil amendments, or other methods to limit the development of resistance.

<u>Nanoparticle formulations enhance biopesticide efficacy.</u> Environmentally friendly biopesticides such as entomopathogenic (insect-killing) nematodes and fungi can control various economically important insect pests such as pecan weevil and peachtree borer. 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, have a research agreement with the Agricultural Research Organization Volcani Center in Israel that is directed toward developing novel formulations for biopesticides based on nanotechnology. ARS and Israeli partners discovered that nanoparticle-based formulations protect biopesticides from ultraviolet radiation and thereby increase pest control efficacy. This technology can lead to improved sustainability in pest management practices. A patent application is being submitted based on the discovery.

<u>Use of Symbiont™ technology for *in vitro* production of therapeutic molecules.</u> Continued activities in the second year of a $15 million 5-year NIFA grant led by ARS researchers in Fort Pierce, Florida, and Ithaca, New York, demonstrated that proprietary Symbiont technology can be used for *in vitro* production of large quantities of therapeutic molecules. The multidisciplinary work conducted on this grant, and as part of a CRADA with the agricultural biotechnology company AgroSource, Inc., demonstrated that symbiont tissue can be cultured *in vitro* for the cost-effective production of molecules that are secreted into the media and continuously harvested. This was demonstrated using fluorescent marker proteins and by production of a special class of antibodies called nanobodies. The collaborating scientists studied the ability to make nanobodies in their Symbiont system because they are also developing nanobodies against effector proteins produced and secreted by the bacterium that causes Huanglongbing (HLB, or citrus greening). These effectors induce HLB disease symptoms in infected citrus plants, and other lines of research indicate that binding antibodies/nanobodies to these effectors prevents disease development. The scientists also demonstrated that functional nanobodies can be produced in plant cells using a gene encoding a nanobody that targets SARS-CoV-2 spike proteins. This nanobody was found to inhibit the interaction of the SARS-CoV-2 spike protein and the surface antigen on mammalian cells that it uses to enter the cell. This work shows that the plant-based Symbiont cells can be used to

produce therapeutic molecules, and current research is underway to evaluate the Symbiont system for producing therapeutic candidates to treat citrus HLB disease.

Discovery of a new class of safer insecticides against yellow fever mosquito. ARS scientists in Beltsville, Maryland, discovered novel compositions of chemicals for repelling, knocking down, and killing adult female yellow fever mosquitoes (*Aedes aegypti*). Methyl benzoate and several analogs have been shown to have spatial repellent properties, knockdown effects, and lethal activity against *Ae. aegypti* when compared with the "gold standard" mosquito repellent, DEET, under laboratory conditions. These compounds are the most promising DEET alternatives and have significant potential for being used to develop new products. They provide improved real-world protection against *Ae. aegypti* as well as other mosquito pests and their vectored human and animal diseases.

A new method to block virus transmission by aphids. Many viruses that infect plants, including the new cotton leafroll dwarf virus infecting U.S. cotton, rely on an insect vector for plant-to-plant spread. ARS scientists in Ithaca, New York, and university cooperators focused on a group of insect-transmitted viruses, the poleroviruses, which cause economically important diseases of vegetables and other crops, including cotton. The scientists determined the structure of a protein on the surface of these viruses that is crucial for the virus to be picked up and transmitted by its aphid insect vector. They also found that this protein could be used to block virus transmission; when the insects feed on this protein they can no longer effectively transmit the virus. The team showed this protein is a powerful inhibitor of virus transmission across three different delivery strategies by blocking entry of the virus into aphid tissues. Moreover, the research revealed that variants of this protein are lethal to the insects. The results show that ARS scientists found a new way to block the transmission of these economically important viruses as well as a new way to kill the insect vectors. The work impacts not only scientists who want to understand these viruses, but also industry and growers who are looking for new ways to help manage these destructive viral pathogens. The transmission blocking protein was also helpful to the team for developing novel diagnostics tools. A collaboration with a company is helping to commercialize a diagnostic kit for cotton growers to detect the presence of a newly emerging cotton polerovirus.

Sucralose added to erythritol controls spotted wing drosophila. Growers requested safer alternative products to control spotted wing drosophila (SWD, *Drosophila suzukii*), a fly that attacks small fruits and cherries, causing more than $500 million in damage. Erythritol is a human-safe non-caloric sugar that is toxic to the fly and is easily sprayed. ARS scientists in Corvallis, Oregon, found that adding sucralose to erythritol enhances its sweetness, causing flies to consume more of it, die more quickly, and lay fewer eggs. Also, sucralose is not metabolized into useable carbohydrates by the insect and causes them to lose moisture. Based on this work, sucralose erythritol formulations are being examined by other scientists in field trials and by companies as an alternative to broad-range insecticides to control SWD.

Genomics uncovers novel genetic basis of resistance to Bt toxin Cry1Ac in the corn earworm. Genetically engineered crops that produce insecticidal proteins from *Bacillus thuringiensis* (Bt) have many benefits and are important globally for managing insect pests. However, the evolution of pest resistance to Bt crops reduces their benefits. As part of Ag100Pest and together with University of Arizona collaborators, ARS researchers in Maricopa, Arizona, produced the first chromosome-level genome assembly of *Helicoverpa zea* (corn earworm), which is a highly damaging pest of U.S. cotton and corn, and discovered a novel gene for resistance to Bt toxin. The findings provide direct application for genomics to detect novel Bt resistance genes.

Gene editing of eye pigmentation genes in Western tarnished plant bug provides direct validation of gene function. The Western tarnished plant bug (*Lygus Hesperus*) is a key pest of cotton ($30 million damage) and other crops such as strawberry ($40 million damage) throughout the western United States and producers need new pest management strategies. Powerful new molecular genetic biocontrol tools are becoming available that can cause species-specific disruption of critical biological processes in pests. However, such genetic biocontrol strategies require expansion of molecular knowledge and tool development in each target pest species. ARS scientists in Maricopa, Arizona, used CRISPR/Cas9 gene editing to disable eye color genes and produce stable *L. hesperus* strains with eye color mutants that differed from wild-type individuals. This work demonstrates that CRISPR gene editing functions in this insect and that eye pigmentation genes are useful for tracking its successful genetic manipulation.

Novel mechanisms of western corn rootworm resistance revealed. Nicknamed “the billion-dollar beetle”, western corn rootworm (WCR), which feeds on maize roots, is the biggest cause of feeding damage to maize in the United States. For control, farmers rely on maize varieties that express one or more insecticidal *Bacillus thuringiensis* (Bt) proteins in the plant’s roots, but the pest is increasingly difficult to manage because it has developed resistance across wide areas of the United States. The molecular mechanisms by which Bt proteins cause death of susceptible WCR larvae remain unknown. ARS researchers in Ames, Iowa, and an international team of collaborators investigated changes in gene expression among Bt-susceptible WCR larvae that consumed maize varieties expressing different types of Bt proteins. In addition to metabolic effects, larvae consuming Bt maize increased expression of genes involved in stress responses, including genes involved in determining if cells should be recycled (apoptosis) or repaired. These findings identified novel genes that help WCR recover from exposure to Bt maize and point to novel mechanisms by which resistance may evolve. This information will help guide university, government, and industry research for evaluating and improving insect resistance management strategies to maintain the effectiveness of Bt maize for the control of WCR.

Deep learning algorithms used to improve detection of the Asian citrus psyllid. The Asian citrus psyllid vectors the pathogen causing Huanglongbing (citrus greening), with devastating effects on the Florida citrus industry. Early detection of the psyllid on young trees and in orchards can facilitate tree protection and targeted control efforts. ARS scientists in Gainesville, Florida, used deep learning methods to identify and characterize differences between male and female psyllid courtship calls. In addition to providing a method to differentiate males and females in infestations, this data has potential for the development of vibration-based control methods of mating disruption. Such a behavior-based control approach can help reduce use of pesticides for management of the psyllid.

Potential biomarkers for port detection of fruit fly eggs and larvae in mangoes. Fruit flies are a critical insect pest to many agricultural products and incur serious commodity and economic damage. Part of the economic damage to the agricultural industry is international shipping restrictions on products infested with fruit flies. ARS researchers in Gainesville, Florida, collaborated with USDA-APHIS and researchers in Kenya on identifying biomarker odors (chemical compounds unique to insect infestation) of important agricultural commerce products and others denoting infestation with insect pests. The identification of these biomarker odors in mango provides USDA-APHIS with important information for finding and removing infested fruits at commerce points, thus helping to control the spread of invasive insect species as well as helping to protect the safety of food products.

Genetic markers reveal new introduction of fall armyworm into Africa. Previous studies by ARS scientists in Gainesville, Florida, demonstrated that only the fall armyworm (FAW) strain specific to corn and sorghum was present in Africa, limiting the potential crops at risk of infestation. New studies from these scientists found evidence for a more recent introduction of a new strain of FAW, indicating that the continent is also at risk for the establishment of a strain specific to millet, alfalfa, and forage grasses. This represents a significantly greater impact of the FAW as a threat to crop production in Africa and thus of heightened concern to African farmers and to U.S. and international aid groups.

New method for tracking the citrus greening pathogen in single insects. Citrus greening disease is the most serious disease of citrus. In the United States, the disease is endemic in Florida and spreading in California and Texas; it is ultimately fatal for infected citrus trees, and there is no cure. The disease comes from plant infection by the bacterium CLas, which is acquired by the Asian citrus psyllid when it feeds on infected citrus trees and then transmits the pathogen to other trees. ARS scientists in Ithaca, New York, developed a method to enrich CLas cells from single psyllid insects for direct genome sequencing. The data generated allowed assembly of CLas genomes from single insects and the identification of differences in CLas DNA sequences that permits strain identification. This method allowed for tracking the strain of CLas from a single insect, which might help trace the origin of new outbreaks.

Transmission of a costly grapevine virus by an insect vector in vineyards. Grapevine red blotch virus (GRBV) is a new virus that infects grapevines in North America, disrupting grape production and vineyard productivity. GRBV is spread in vineyards by a tiny insect called the three-cornered alfalfa hopper. Free-living grapevines, frequently found in areas surrounding commercial vineyards where cultivated wine grapes are grown, can serve as a hiding place for GRBV. ARS scientists in Ithaca, New York, and university partners showed that insects transmit the virus more efficiently into free-living grapes as compared to the cultivated wine grapes and that both types of plants—free-living and wine grape—can serve as sources of virus that is spread by the alfalfa hopper. The results suggest that management of the virus in free-living grape vines in areas around a commercial vineyard may be one way to reduce virus spread into the vineyard by the insect vector.

Field-evolved resistance of western corn rootworm to Bt corn. Transgenic (Bt) corn expressing the Cry3Bb1 protein has been used to control western corn rootworm (WCR) across the U.S. Corn Belt, but the WCR has now evolved resistance to the protein. Corn hybrids that contain Cry3Bb1 and the Cry34/35Ab1 protein have been increasingly used to counter WCR resistance to Cry3Bb1 alone, but assessments are needed to determine the susceptibility of WCR populations to that pyramid. In laboratory tests, ARS scientists in Brookings, South Dakota, and University of Nebraska scientists found that WCR collected from northeast Nebraska (where both corn hybrids were planted) had some level of resistance to both hybrids; most WCR populations exhibited incomplete resistance to Cry3Bb1 + Cry34/35Ab1 corn, and complete resistance to Cry3Bb1 maize. The results demonstrated a regional pattern of WCR resistance in areas characterized by long-term continuous corn production and associated planting of Cry3Bb1 hybrids. The resistance is important to corn farmers and pest management practitioners because it suggests that the use of a multi-tactic integrated pest management approach is needed in areas of continuous corn production to slow or mitigate resistance evolution to corn hybrids.

Prediction model determines population dynamics of sugarcane aphids. Sugarcane aphid is a sorghum pest that depends on windborne migration to infest and damage sorghum fields at latitudes north of mid-Texas. ARS scientists in Stillwater, Oklahoma, developed a computer-based mathematical model to simulate sugarcane aphid within-field population growth and northward migration. The model captured salient features of population dynamics and migration, and testing showed accurate predictions of within-field population growth and timing of the first occurrence of migrant aphids at all test sites north of central Texas. Sorghum producers throughout the U.S. Great Plains can use this model to track sugarcane aphid infestations and accurately predict populations in almost real time.

Insect herbivory influences extrafloral nectar content in cotton. Cotton plants produce extrafloral nectar on leaves and reproductive structures to attract parasitoids and predatory insects such as ants, which in turn protect the plant against herbivorous insects. Cotton generally produces more nectar in the bracteals of fruiting structures than in leaves, but when plants are attacked by herbivores, nectar production in leaves increases substantially in response. ARS researchers at College Station, Texas, and Texas A&M University collaborators demonstrated that foliar herbivory significantly increased the sucrose content of bracteal nectar, while glucose and fructose content remained unchanged. Although sucrose content has been shown to influence ant foraging behavior in other crops, no difference in ant foraging behavior was observed in the field using mock nectar solutions varying in sucrose content. These findings raise new questions regarding the evolutionary relationship between ants and nectar production in cotton and represent a significant contribution to cotton host-plant relationship research that may be exploitable in developing new cotton types that are resistant to damage from arthropods and other pests.

Attack rates and consumption of pests in perennial polyculture. The agricultural landscape of the U.S. Midwest has been simplified over the past 100 years with large portions of land converted primarily to monocultures of corn and soybean. ARS scientists in Brookings, South Dakota, and University of Illinois colleagues evaluated how increasing plant diversity by shifting from monoculture to polyculture affects beneficial arthropod such as ants throughout an annual cycle. The scientists found that polycultures of clovers, grasses, and woody perennials harbored more ant species than conventional monocultures. Increased diversity was correlated with significantly higher attack rates and consumption of pests. The important findings provide U.S. Midwest farmers with strong evidence that Midwestern landscapes can be deliberately modified to support beneficial insects like ants, while still producing economically viable alternative commodities.

Feeding apparatus of the southern green stink bug. Stink bugs use needle-like mouthparts known as stylets to feed on various plant structures, and their feeding can result in the transmission of plant pathogens. The mouthparts are composed of an outer pair of stylets that encompasses an inner pair of stylets to form the feeding apparatus. Although the external characteristics of the stylets are known, the internal components, including nerve bundles, have not been reported. ARS researchers in College Station, Texas, used transmission electron microscopy to obtain high resolution images of the internal components of the stylets. The images showed a counterclockwise rotation of the feeding apparatus throughout the entire length of the mouthparts. The images also revealed two types of nerve bundles within the outer stylets, suggesting the presence of nerve bundles within the inner stylets, which in previous studies were described to be void of nerves. These findings improve the understanding of the stink bug needle-like feeding apparatus and, more importantly, provides additional insight into its relationship with the insect's internal anatomy, feeding behavior, and pathogen transmission.

Newly discovered virus infects potato psyllid. Potato psyllid is a potato pest and the vector of the pathogen that causes zebra chip disease. New methods that are safe for consumers and the environment are needed to effectively control potato psyllid and zebra chip disease. ARS scientists in Wapato, Washington, in collaboration with researchers at the University of Idaho, discovered a new virus named Bactericera cockerelli picorna-like virus (BcPLV) that infects potato psyllids. BcPLV, along with a related virus that infects the Asian citrus psyllid, form a new genus that the researchers provisionally named Psylloidivirus. This discovery will allow researchers to test whether BcPLV provides biological control of potato psyllid to prevent zebra chip disease and reduce the need for chemical insecticides.

Pyrethrin combined with an organic, food-grade coating protects cherries against western cherry fruit fly. Western cherry fruit fly is a quarantine pest of sweet cherries in western North America, where there is zero tolerance for fly-infested fruit. Organic control options for the fly are limited, and new management tools are urgently needed. ARS scientists in Wapato, Washington, and Lincoln University (New Zealand) collaborators studied how combining organic insecticides (pyrethrins) with an organic, hydrophobic food-grade coating affected egg laying by the western cherry fruit fly. They found that the combination of these two organic products reduced egg laying by flies in cherries nearly 100 percent more than using pyrethrins alone. These results provide cherry farmers a new organic-based method to control the western cherry fruit fly.

Heat treatments for killing apple maggot in organic yard waste. Apple maggot fly is a key quarantine pest of apple, and Washington State imposes strict guidelines on the movement of apple maggot-infested materials to exclude this pest from apple growing regions of the state. Movement of apple maggot to fly-free regions occurs when yard waste from western Washington is transported to commercial composting facilities in eastern Washington. ARS researchers in Wapato, Washington, in collaboration with Engineered Compost Systems (Seattle, Washington), developed heat treatments to kill 100 percent of apple maggots in yard waste. Government agencies are now using this information to develop protocols to eliminate the movement of apple maggot in organic waste to fly-free apple growing regions of Washington.

New biological control fungi for soybean cyst nematode and plant pathogens. The soybean cyst nematode (SCN; *Heterodera glycines*) is the largest cause of soybean yield loss in the United States and worldwide. This pathogen is particularly difficult to manage because most nematicides that were historically effective are highly toxic to both humans and wildlife and have been banned. ARS scientists in Ithaca, New York, obtained funding to isolate and characterize the fungi in soybean cyst nematode cysts and suppressive soils. Results of greenhouse testing of nematode egg-parasitic fungi showed that several isolates were as effective as commercial fungal biocontrol products (DiTera ® and MeloCon® WG) at lower application doses and were more effective than bacterial products (CLARIVA® pn and Poncho/VOTiVO®). The new isolates show potential for use as biocontrol agents or biopesticides for more sustainable integrated pest management of SCN. *Fusarium virguliforme*, the causal agent of Soybean Sudden Death Syndrome, often co-occurs with SCN in soybean fields and may be associated with cysts of the SCN or introduced through wounding by the nematode. Scientists analyzed the genetic diversity of *F. virguliforme* isolates across the U.S. Midwest to identify three major genetic groups, one of which has higher genetic diversity that may enable it to adapt and spread as an invasive pathogen.

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

Development of synergistic controls for the navel orangeworm. The navel orangeworm is the most important insect pest damaging California’s $8 billion almond and pistachio crops. Mating disruption is widely used for this pest, but industry wants to further expand the role of this environmentally friendly technique in overall management of navel orangeworm. ARS scientists in Parlier, California, and Trece, Inc. collaborators used a 10-year data set from 2,400 acres of commercial almonds to compare results from three treatment controls: mating disruption only, insecticide only using a reduced risk egg and larva pesticide, or both. Across the entire study, the two treatments together significantly reduced navel orangeworm damage, and this trend was evident in 9 of the 10 years in the study. Demonstration that mating disruption enhances control with a widely used insecticide will improve adoption of mating disruption and reduce insecticide use in the long run.

Methods developed to control invasive and quarantine horticultural pests. ARS researchers in Parlier, California, developed novel postharvest methyl bromide fumigation to control codling moth in more than 50 new varieties of fresh plum exported from California to Japan, valued at $12 million annually. A novel postharvest methyl bromide fumigation was also developed to control codling moth infesting in-shell walnuts exported from California to Korea valued at $20 million annually. The research contributed to market retention and/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 their respective counterparts in foreign governments to maintain trade.

Effects of nitric oxide fumigation against ham mites. Ham mites (*Tyrophagus putrescentiae*) are important pests on aged hams and stored products, such as cheeses, nuts, dried fruits, spices, and semi-moist pet foods. Methyl bromide fumigation has been the main control treatment in the past, but this fumigant is being phased out worldwide because of its ozone depleting impact on the environment. Nitric oxide (NO) is a newly discovered fumigant that has demonstrated safety and efficacy against a variety of insect pests. ARS researchers in Miami, Florida, and Salinas, California, in collaboration with Kansas State University, developed fumigation protocols using NO to control ham mites on ham meat under ultra-low oxygen conditions. The results showed that complete control was achieved for all mite life stages; eggs were the most tolerant stage, but 100 percent mortality was obtained after 48- and 24-hour treatments at 0.5 and 1.0 percent NO concentrations, respectively. NO fumigation, a safe and effective treatment, provides a potential alternative to methyl bromide fumigation for postharvest pest control.

A novel oviposition deterrent for oriental fruit fly identified. Oriental fruit fly (OFF) is one of the most destructive pests infecting agricultural produce. Current OFF prevention programs in the mainland United States focus on control and surveillance of male flies, which is not effective for mitigating the impact caused by invading female flies. Protein bait sprays have been extensively used to protect fruit infestation by OFF, but resistance development is an issue. ARS researchers in Hilo, Hawaii, identified natural product-based oviposition deterrents and identified four key chemicals that can significantly reduce fruit infestation when applied on fruit. An invention disclosure was submitted in March 2022. Prototype sprayable formulations of the active ingredients are being formulated through a CRADA for field testing in 2022.

A novel method to repel spotted wing drosophila to protect fruit. Current control methods to reduce fruit infestation by spotted wing drosophila are based on the constant use of pesticides, which may not be sustainable due to resistance, cost, and negative impact on environment and beneficial insects. ARS researchers in Hilo, Hawaii, identified 2-pentylfuran, a novel spatial repellent that can reduce spotted wing drosophila infestation on fruit by 50 percent without the use of pesticide. 2-pentylfuran is a compound that is generally regarded as safe and will be a safe alternative to pesticide spraying. A patent was filed in February 2021 and a prototype puffer dispenser was developed via CRADA for field testing.

Repellents for pest ambrosia beetles. Redbay ambrosia beetle and tea shot-hole borer are vectors of laurel wilt and Fusarium dieback, respectively, two fungal diseases of avocado, woody ornamentals, and native forest trees. Incorporating a repellent into pest management programs may reduce the incidence of these diseases. ARS researchers in Miami, Florida, identified piperitone as a new beetle repellent and compared its efficacy to two other repellents, verbenone and a-farnesene. Beetle captures in traps baited with lures were compared to captures in traps containing lures plus a repellent (a push-pull design). The researchers determined that piperitone and verbenone were equally effective, reducing captures 50-70 percent for 10-12 weeks. Since piperitone is less expensive than verbenone, the standard beetle repellent, these studies identify an economical alternative for management of both pests in commercial avocado groves.

Improved monitoring and control of invasive stored product insects. Although not established in the United States, the khapra beetle and larger grain borer are of significant concern to stakeholders. The larger grain borer could expand its range northward from Mexico into the southern United States under climate change scenarios, and the khapra beetle is a quarantine species that is routinely intercepted at ports. ARS researchers in Manhattan, Kansas, identified a novel reduced-risk insecticide that induced significant mortality and reduced mobility of larger grain borers when applied to bulk grain. Moreover, the new formulation is more cost-effective than existing formulations because it has lower concentrations of the active ingredients but retains the same efficacy. In addition, trap placement and efficacy were optimized for monitoring the khapra beetle. Currently, traps are placed on vertical surfaces, which likely reduces the number of larvae caught in these traps and can cause underestimations in population size. However, applying these traps to rougher surfaces, such as plywood, sheetrock, tile, cement, tape, and netting, improved the climbing ability of larvae and thus, their trap captures. Collectively, these studies provide new methods for monitoring and controlling these invasive insects.

New integrated pest management tactics for phosphine resistant stored product insects. Stored-product insect pests with phosphine resistance are increasing in abundance and distribution around the world. Phosphine is one of the most commonly used fumigants in large warehouses and food facilities for insect control, and the emergence of resistance threatens its efficacy, which has become a key stakeholder concern. Long-lasting insecticide-incorporated netting (LLIN) and packing material containing insecticides have been effective against several species of stored product insects. ARS researchers in Manhattan, Kansas, evaluated the efficacy LLIN and packaging materials containing four different active ingredients (deltamethrin, permethrin, indoxacarb, and dinotefuran) against phosphine -resistant populations of lesser grain borer and red flour beetle. Overall, all compounds caused significant mortality in phosphine-resistant strains of both species, with the exception of indoxacarb for red flour beetle. Using bags and netting impregnated with insecticides provides new tools to prevent and reduce infestations of stored product insects, including those that have evolved resistance to phosphine.

<u>Nine potential repellents for invasive spotted wing drosophila pests.</u> In the United States, the invasive spotted wing drosophila is a serious pest of fruits, causing more than $300 million in damages annually. Because their eggs and larvae are enclosed within infested fruit, they are shielded from insecticide sprays, which makes them difficult to control using conventional methods. As an alternative or supplement, odors can be used to manipulate the pest's behavior in the field. ARS researchers in Gainesville, Florida, and Rutgers University collaborators discovered that flies avoided fruit infected with a specific pathogen. By studying the odors emitted from pathogen-infected fruit and examining pest responses to these odors in the laboratory, they identified nine previously unreported chemical repellents that can potentially be used to prevent flies from damaging crops. This technology has the potential to be developed for improving crop protection and reducing insecticide use.

<u>A companion crop reduces tomato infection by root knot nematode.</u> Plant parasitic nematode infections of cash crops cause an excess of $125 billion in annual crop damage worldwide, and these nematodes are widely regarded as the most damaging biotic crop stressor with no real viable management option. In collaboration with scientists from Nairobi, Kenya, ARS researchers in Gainesville, Florida, reported a novel and sustainable approach to an intractable pathogen, the root knot nematode (*Meloidogyne incognita)*. The scientists used the wild growing, readily available vegetable black-jack (*Bidens pilosa*) as a companion crop to deliver root exudate to mask/interfere with the root knot nematode-tomato interaction. Achieving safe and effective nematode control is a key part of the movement toward environmentally sustainable agriculture.

<u>Releases of a tiny wasp to control spotted wing drosophila.</u> Organic growers have one primary pesticide to control spotted wing drosophila (SWD, *Drosophila suzukii*), a major pest of fruit crops, and are interested in biological control options. Releases of parasitic wasps that attack the fly may be helpful in protected environments, and where movement of pesticide spray equipment is limited. ARS scientists in Corvallis, Oregon, and Albany, California, as well as University of Minnesota, tested releases of the parasitic wasp *Pachycrepoideus vindemiae* in caneberries. Modest release rates of the wasp combined with an augmentorium (a finely meshed structure containing waste fruit that allows parasites to escape but traps the flies) resulted in higher parasitism rates. Similarly, the parasitic wasp *Ganaspis brasiliensis*, a key natural enemy of SWD in Asia, was provided and released, with the aim of permanent establishment at many field and landscape reservoir sites in 17 states, by ARS scientists in Albany, California; Corvallis, Oregon; Wapato, Washington; Newark, Delaware; and university cooperators across the country. Such results, when combined with other techniques, are being developed into environmentally safe alternatives to pesticides for fruit protection.