

**United States Department of Agriculture
Agricultural Research Service
National Program 304**



Crop Protection and Quarantine

FY 2023 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 2023. The report below is not intended to be a progress report describing all research conducted during the 2023 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).

Component 1 – Systematics and Identification

High quality boll weevil reference genome. The boll weevil was first detected in Texas in 1892 and began spreading throughout the U.S. Cotton Belt and has often been considered the most destructive pest of commercial cotton in the United States. Although national eradication efforts have essentially eliminated this insect pest in the United States, eradication progress in South Texas and Tamaulipas, Mexico, have been at a standstill for the past decade. As a result, remaining boll weevil populations continue to pose a threat to previously eradicated areas. Growers are concerned that boll weevils may develop resistance to insecticides, so safe and effective alternative boll weevil management strategies are needed. As part of the Ag100 Pest Initiative, ARS researchers in College Station, Texas, sequenced, assembled, and annotated the boll weevil genome. The high-quality reference genome will be foundational to gaining a better understanding of boll weevil biology and ecology, genetic diversity, and for identifying novel genes that can be targeted or exploited with gene disruption technologies (e.g., CRISPR, RNAi) to develop alternative target-specific boll weevil management strategies that are safe and effective.

Frequent and efficient harvests to control coffee berry borer. Coffee is the second most economically important crop in Hawaii, and green and roasted coffee is valued at about \$175M. Coffee berry borer (CBB) infests the coffee seed and reduces coffee yields and quality. ARS scientists in Hilo, Hawaii, collaborated with coffee growers on quantifying the costs and benefits associated with sanitation picks, standard harvesting, and strip picks, and on estimating total costs of controlling CBB using pesticides on Hawaiian coffee farms. They found cultural control-focused management consisting of fewer pesticide sprays and more frequent sanitation and harvesting are effective and economically viable alternatives to regular pesticide applications to control CBB. Chemical control costs were found to be 55 percent lower, coffee cherry yields increased 3000lbs/ac, and the net benefit of frequent harvesting were 48 percent higher under the proposed integrated pest management. This information has been disseminated to coffee growers in Hawaii by two grower association newsletters, a mailing list, an Entomology Today blog post, farm visits, and a consultant seminar, and was shared with University of Hawaii Extension.

Predator and plant feeding stinkbugs react differently to an insecticide. Stink bugs (Pentatomidae) are one of the few “true bugs” that contain both plant feeders and predators and comprise a range of invasive agricultural pests and natural enemies of great economic importance. Scientists in the Sino-American

Biological Control Laboratory, an ARS-associated lab in Beijing, China, examined the acute toxicity of the insecticide imidacloprid to the plant feeding stinkbug *Halyomorpha halys* and the predator stinkbug *Arma chinensis*. They found the plant feeding stinkbug is significantly more sensitive to imidacloprid than the predator, and female adults of both species showed higher insecticide resistance than male adults. In addition, reduced reproduction performance indicated that predator stinkbug recovery from imidacloprid-triggered knockdown was achieved at the cost of fitness. Results based on acute toxicity and molecular analysis indicate that imidacloprid is effective against the plant feeding stinkbug, but potentially safe for the predatory stinkbug when employed as a biological control agent. This is of particular interest for integrated pest control.

Reduced application density for male annihilation technique against oriental fruit fly. As a result of ARS research conducted by scientists in Hilo, Hawaii, APHIS, and an Australian areawide project in Malaysia revised their guidelines on application densities needed for using the male annihilation technique (MAT) against oriental fruit fly. Suggested application densities were reduced by half, which will result in large cost savings for material and labor and reduce the environmental impact of the insecticide used in conjunction with the attractant in MAT. For a high-detection year in California, such as 2015, the change is estimated to result in \$380,000 annually in direct savings.

Hyper Diverse Subfamily of Leaf Beetles. Phylogenetic research confirmed three tribes within a hyper diverse subfamily of leaf beetles. ARS researchers in collaboration with scientists in Canada, China, and India, generated and analyzed a genomic dataset of 925 nuclear loci for 100 genera of Chrysomelidae. The resulted classification placed the enigmatic, conifer root-feeding, Synetinae as separate from subfamily Eumolpinae (known to feed on the roots of a various angiosperms). The following tribes were confirmed within Galerucinae: Alticini with 700 genera and 10,000 species, Galerucini with about 500 genera and 6,000 species and Serraticollini as evolutionary sister taxon to all remaining Galerucinae with one genus and 60 species. Memberships in the tribes Alticini and Galerucini were corrected by transferring incorrectly placed genera between them. The study will be useful to biological control workers, evolutionary biologists, ecologists, and anyone interested in plant feeding beetles.

Cucumber beetle pheromone for other vegetable pests. Using a pest's own pheromones is a powerful tool for biological pest management control, but many pheromones are still unknown, and many known pheromones have not been tested for their potential pest management application. ARS scientists in Beltsville, Maryland, and collaborators from University of California-Davis, Virginia Tech, Cornell University, and the University of New Hampshire demonstrated that the male-produced aggregation pheromone of the striped cucumber beetle, which was synthesized by ARS, is also attractive to four other cucurbit (vegetable) pests: spotted cucumber beetle, squash bug, horned squash bug, and western striped cucumber beetle. This attraction enables developing traps, baits, or other attract-and-kill tactics useful in integrated pest management for cucurbit pests and for future commercialization.

Systematics of true fruit flies. Research was published on the systematics of true fruit flies, including new host plant data and descriptions of 46 previously unknown species from Bolivia, Colombia, Suriname, French Guiana, and Brazil, belonging to the largest and most economically important group of fruit flies in the American tropics (*Anastrepha*). Previously unknown larval characters were described for nine species. An evolutionary analysis of the drivers of worldwide global fruit fly invasions was carried out and further development of an online identification tool for the more than 350 species of *Anastrepha* was developed to facilitate identifications by other scientists. A subsample of species was analyzed via NextGen sequencing methods towards a phylogenetic analysis to determine evolutionary relationships of the entire family of highly damaging flies. Additional names, distribution, and host plant data for fruit flies were compiled to be added to the comprehensive database available via APHIS-CPHST web site; this information

is critical to APHIS-PPQ and other regulatory agencies to prevent the spread of pest species into the United States.

Sweetpotato clones with improved insect resistance and weed tolerance. Sweetpotato growers are concerned about managing weeds and insect pests because of limited options and crop tolerance or resistance to weeds and insect pests offer an effective sustainable solution. ARS researchers in Charleston, South Carolina, and Clemson University collaborators investigated how weed-free intervals and sweetpotato clones affected weed counts for naturally occurring weed species, storage root yield, and insect resistance to major sweetpotato pests. The researchers identified two sweetpotato clones (USDA-17-037 and USDA-17-077) that had low weed counts, broad insect resistance, and the highest yields among those tested. This research demonstrates that developing sweetpotato cultivars that are competitive with weed interference through novel plant architecture (erect growth habit) and with resistance to insects is useful for developing an effective pest management strategy with particular benefit for organic and sustainable growers.

Component 2 – Weeds

Signaling mechanisms involved in weed-induced crop yield loss. Crops perceive weed-generated signals through changes in light quality and through volatile and soil-soluble chemicals. Scientists need information about how these signals affect plant genes to manipulate crop responses to weeds and mitigate weed-induced yield losses. ARS scientists in Fargo, North Dakota, conducted time course studies to examine weed-induced changes in corn growth and gene expression when exposed to soil soluble and light quality signals separately or in combination. Soil soluble signals had a greater impact on corn growth than light quality signals and affected leaf photosynthesis and root cell wall production. The light quality signal had no consistent impact on leaves but affected protein turnover and cell growth in roots. The research implicated certain gene networks and protein complexes known to regulate the balance between corn plant defense and growth when challenged by weeds. This work will help breeders create more weed-tolerant crops by suggesting ways to manipulate crop responses to weed-generated signals.

Biological agents to control invasive cogon grass. Cogon grass (*Imperata cylindrica*), a federally regulated noxious invasive grass with a worldwide distribution, is considered one of the worst invasive species in the United States, causing economic and ecological damage that disrupts forestry, agriculture, rangelands, and natural ecosystems. Increased management options such as mechanical and chemical controls are expensive, and time consuming and new strategies are needed to reduce the spread of cogon grass. Effective biological control agents have not been available to date, but scientists in the Australian Biological Control Laboratory, an ARS-associated lab, discovered a diverse range of potential biological control agents that might be specific to cogon grass and that are immediately available for shipment to U.S. quarantine facilities. This will enable stateside ARS researchers to begin testing for efficacy and their safe release to interested parties and agencies.

Regions of the *Camelina sativa* genome associated with important agronomic traits. Breeding for desired agronomic traits is critical for improving cropping systems. ARS scientists in Fargo, North Dakota; Clay Center, Nebraska; and Stoneville, Mississippi, collaborated with North Dakota State University scientists on sequencing and identifying genomic regions in a camelina population developed from crossing a winter biotype with a summer biotype. They were looking for genomic associations with freezing tolerance, flowering time, and seed size, and identified specific markers within the 20 assembled chromosomes associated with these important agronomic traits. These outcomes will help breeders improve important agronomic traits in oilseed cash crops used for developing cropping systems suitable for colder climates and soils.

First biocontrol agent to control common crupina released in field studies. Rangelands and grasslands make up 30 percent of U.S. land use and are vital to livestock production and ecosystem health. Controlling invasive weeds in these rugged environments is challenging and incurs U.S. costs of \$6 billion annually. Common crupina is a foreign weed that has invaded thousands of acres of grasslands in the U.S. West, crowds out nutritious plants that provide food for grazing livestock and leads to conditions that result in soil erosion. Chemical-based weed control methods are often impractical because of the large size of invaded regions. ARS scientists in Frederick, Maryland, and university and tribal collaborators tested a new non-chemical method to control common crupina. The approach relies on using a fungus that causes a leaf spot disease on only common crupina and within weeks of being released at the test sites, the fungus successfully infected common crupina. The results of this research suggest this new biological approach may be a valuable tool to reduce common crupina invasions in rangelands and grasslands and benefit private, public, and tribal land managers.

New herbicide development for weed management. The triketone class of herbicides plays an important role in controlling weeds, especially in corn, soybean, and wheat crops. The main herbicides in this class are mesotrione and sulcotrione, which are analogs of the allelochemical leptospermone from the bottlebrush plant. However, the degradation products of mesotrione and sulcotrione can negatively affect aquatic plants and microorganisms. The benzoic rings resulting from the degradation of these herbicides are responsible for the toxic effects. To develop more environmentally friendly triketone herbicides, ARS scientists in Oxford, Mississippi, and University of Mississippi collaborators made a series of structurally related triketone analogs originating from malonic acid, a naturally occurring compound found in many fruits and vegetables. Bioassay results indicated that these newly synthesized compounds exhibited strong herbicidal activity, but unlike mesotrione and sulcotrione, they lack benzoic rings. The new chemical entities (keto-diesters) provide a new class of herbicides and their mode of action is likely similar to that of mesotrione and sulcotrione. In addition, unlike commercial triketone herbicides, several keto diether-based compounds developed in this project can reduce potential leaching in plant leaves. The ARS National Chemical Patent Committee approved the invention disclosure entitled "Derivatives of natural triketones and their uses" in 2022.

Improving grass seedling establishment in rangelands. Establishing grasses to restore degraded rangelands is critical but difficult to accomplish using only seed-based methods. ARS researchers in Burns, Oregon, developed techniques for harvesting, excavating, and storing buds of native plant species, and developed methods for successfully establishing Sandberg's bluegrass and tufted hairgrass, two important native plants, from crown buds during restoration. Using grass buds in rangeland restoration will be useful to state and federal land management agencies, producers, conservation groups, and anyone attempting to restore these species in degraded and invaded rangeland.

Software tool for processing accurate genome assembly. Genome sequencing and assembly represents a large component of life science research programs. This assembly is the foundation of both exploratory and hypothesis-based studies, and highly accurate genome assemblies are necessary to conduct downstream analyses. ARS researchers in Hilo, Hawaii, have developed a widely adopted software program called HiFiAdapterFilt that facilitates genome assembly by removing erroneous sequencing artifacts to prevent their integration into genome assemblies. HiFiAdapterFilt has been used and cited in high-impact studies such as the Human Pan-genome and provides a valuable tool that facilitates accurate genome assembly.

Genomic technology for whitefly management. The sweetpotato whitefly is a globally important crop insect pest that is difficult to manage, even with insecticides. The use of RNA interference (RNAi) technology is a strategy for pest control that scientists are studying for several pests. Collaborative

research between the University of Georgia and ARS researchers in Charleston, South Carolina, found that the Dnmt1 gene serves an essential role in the reproduction of female whiteflies; it affects whitefly egg production and the ability of the eggs to hatch. These findings will aid scientists in the development of a RNAi-based pest management strategy for whiteflies and other pests.

Component 3 – Insects and Mites

Plant-based peptides block insect transmission of the citrus greening disease bacterium. Citrus growers need effective strategies for managing citrus greening disease, also known as Huanglongbing (HLB). Growers are highly concerned about the presence and likely spread of HLB within California because of the mobility of the Asian citrus psyllid (ACP), the insect vector of citrus greening in the United States. In Florida, the disease is endemic, and growers need treatments to mitigate infection in existing groves and prevent transmission into new plantings. Organic growers also need HLB management strategies that do not rely on synthetic chemicals. ARS scientists in Ithaca, New York, discovered plant-based peptides that can thwart the spread of the HLB bacterium by the ACP. This collaborative work was conducted by a team of USDA, university, and industry partners and was funded by a CRADA with a small agri-business in Florida and a grant from the California Citrus Research Board. A patent has been filed and a preprint describing the peptides is available. These plant-based peptides have different killing modes of action that could effectively control citrus greening and prevent ACP from transmitting the pathogen to citrus trees.

Therapeutic genes for citrus trees infected by huanglongbing. Huanglongbing (HLB) has reduced commercial citrus production in Florida more than 70 percent and threatens the citrus industries in Texas and California. There is currently no cure for this disease, and to save the citrus industry, a solution must be delivered to infected trees in citrus groves. The bacterium *Agrobacterium tumefaciens* was used to develop small, localized growths (galls) that were genetically engineered to produce molecules that potentially could confer tolerance to the host citrus tree. The engineered galls are called symbionts because they produce molecules that provide therapeutic benefits to their tree hosts and the therapeutic molecules produced by the symbiont were demonstrated to move systematically in the tree. This new ‘symbiont’ strategy could mitigate some regulatory concerns about the release of genetically engineered crops because the genetically engineered component is the symbiont, not the tree. This technology would be applicable on any plant that *Agrobacterium* could infect and form a gall.

Molecular tool to identify boll weevils. The boll weevil has been eradicated from the United States, except for the southernmost portion of Texas, but eradication programs in cotton-producing states continue to operate pheromone traps to detect possible boll weevil re-infestations. These traps occasionally capture similar looking weevils such as the thurberia weevil, which is a variant of the boll weevil but is not a pest of commercial cotton. Non-pest weevils misidentified as boll weevils could lead to unnecessary and costly mitigation efforts, while misidentifications could delay or preclude necessary remedial actions. ARS researchers in College Station, Texas, partnered with university and Animal and Plant Health Inspection Service (APHIS) colleagues on developing a single nuclear polymorphism (SNP)-based assay that can rapidly and accurately determine whether a weevil is a boll weevil or a thurberia weevil. This molecular tool was recently adopted by APHIS and is currently being used to confirm the identity of suspect boll weevils captured in traps or intercepted at U.S. ports of entry.

Discovery of a new virus infecting upland cotton. The United States is the world’s third largest cotton producer and the leader in cotton exports. Cotton leafroll dwarf virus (CLRVD) is an emerging RNA viral pathogen of cotton transmitted in the field by the cotton aphid. Viral symptoms in the field have been challenging to understand and pinpoint to CLRVD alone. ARS scientists in Ithaca, New York, hypothesized that other plant viruses may be co-infecting cotton with CLRVD. ARS scientists and university colleagues

discovered a new DNA virus infecting cotton growing in Mississippi and tentatively named the virus cotton virus A (CotV-A). Similar to other DNA plant viruses, they discovered that copies of the CotV-A genome are inserted into the genome of upland cotton, which may give rise to virus infection, so the team developed a method to distinguish between the real CotV-A infection and copies of CotV-A in the cotton genome. The cotton industry was informed about the new virus and a paper has been published. Additional research is needed to determine whether CotV-A can cause detrimental effects in cotton production, if the virus copies inserted in the upland cotton genome can initiate a real virus infection, or whether the virus is transmitted by insects.

Electronic cold pasteurization for in-shell weevil control. Pecan weevil is typically an in-season pest that is managed by controlling adult pecan weevils in orchards. Nonetheless, controlling weevils in orchards is not always optimal and can lead to pecan nuts that are infested with pecan weevil larvae. When larvae persist in the nut after harvest, distributing pecan nuts to shelling facilities and marketing venues can also transport pecan weevils to new areas. ARS scientists in Byron, Georgia, showed that using electronic cold pasteurization to treat pecan nuts infested with pecan weevil larvae can kill larvae within nuts. This process could alleviate quarantine concerns regarding the movement of pecan nuts potentially infested with pecan weevil larvae.

A new system for farmers to grow eco-friendly bioinsecticides. Beneficial nematodes (small round worms) are environmentally friendly bioinsecticides. These nematodes are produced commercially and used to control a wide variety of economically important pests, but they can be expensive. ARS scientists in Byron, Georgia, and Madison, Wisconsin, developed a novel system that enables farmers to grow their own beneficial nematodes. The process is sustainable and does not require any outside input or scientific equipment. The team published details on the system in a special collection of scientific articles on the mass production of beneficial organisms and some farmers have already started to adapt a modified version of the system.

Plants to the rescue in the next global pandemic. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the COVID-19 pandemic and is related to SARS-CoV-1 and Middle Eastern Respiratory Syndrome (MERS)-CoV, which emerged in humans in 2003 and 2012, respectively. COVID-19 disease results in a range of outcomes, ranging from asymptomatic infection to death. To date, global vaccinations for SARS-CoV-2 protection are underway, but additional treatments are needed to prevent infection among naïve and even vaccinated individuals. ARS scientists in Ithaca, New York, and Fort Pierce, Florida, together with a CRADA partner, demonstrated that plants can produce nanobodies, which are small functional molecules that interfere with molecular interactions required for virus spread in humans. Producing plant-based nanobody therapies to control virus spread represents a promising new development in mitigating the COVID-19 pandemic.

Development of a method to track insect dispersal. Tracking dispersal patterns in agroecosystems is essential for efficiently managing arthropod pests and conserving natural enemies and pollinators. ARS researchers in Maricopa, Arizona, developed and refined a method to tag key cotton pests (lygus and whitefly), natural enemies, and pollinators with a fluorophore that glows brightly under ultraviolet light. The key features of this novel marking procedure are that they are inexpensive, persistent, easily detected, do not affect insect behavior, and work equally well on large and small insects (e.g., whiteflies and parasitoids). The novel fluorophore marking method will expedite future dispersal research on whiteflies and various other pest and beneficial arthropod species.

Improving gene editing methods in fruit flies. Fruit fly pest species are an important agricultural problem that would benefit from improvements in nonchemical control methods, including sterile insect technique

(SIT). ARS scientists in Gainesville, Florida, demonstrated that the wampa and Prosalpha6T genes were verified as good targets for adult male sperm-specific transcription. These will provide alternative genomic target sequences for CRISPR/Cas9 gene editing that can result in male sterility in drosophilid and tephritid fruit fly pest species through this precision-guided SIT method. The promoter sequences of these genes could also be used for sperm-specific fluorescent protein marking (to identify females mated to field-released males) and to drive conditional lethality systems for male sterility and female lethality. These constructs will facilitate the development of more efficient SIT methods.

Genome assembly for *Bacillus thuringiensis* (Bt) resistant corn earworm (CEW). CEW larvae feed on maize kernels in the ear, which can result in economic damage and create a gateway for pathogens that produce toxic aflatoxins to enter the grain. In the United States, CEW has developed widespread resistance to several Bt proteins in hybrid maize used for pest control, so preventing ear damage in maize increasingly difficult. ARS researchers in Ames, Iowa, and other ARS Ag100Pest Initiative collaborators assembled the genome from a strain of CEW resistant to the Bt protein Cry1Ac. This genome assembly is comprised of intact chromosomes and is at a level of completeness not currently common for insect pests. Gene coding regions predicted for this CEW genome are the most complete reported to date. These genomic resources generated by the Ag100Pest Initiative will provide university, government, and industry scientists with a powerful and dynamic tool for evaluating genomic changes that contribute to development of Bt resistance in pest insect populations.

Increase in global resistance to genetically engineered *Bacillus thuringiensis* (Bt) crops. Transgenic plants producing insecticidal proteins from *Bacillus thuringiensis* (Bt) are grown widely to control pests, but the evolution of insect resistance has reduced the efficacy of Bt crops. ARS scientists in Maricopa, Arizona, and University of Arizona collaborators analyzed global resistance monitoring data for the first 25 years of Bt cultivated crops, including corn, cotton, soybean, and sugarcane. A total of 73 cases were examined including 24 pest species from 12 countries with cases of practical resistance arising from 3 in 2005 to 26 in 2020. Practical resistance was documented in some populations of 11 pest species, collectively affecting 9 widely used crystalline (Cry) Bt toxins in 7 countries. Conversely, 30 cases reflect no decrease in susceptibility to Bt crops in populations of 15 pest species in 9 countries. The remaining 17 cases provide early warnings of resistance, which entail genetically-based decreases in susceptibility without evidence of reduced efficacy. Numerous factors that can lead to sustained susceptibility to Bt crops were reviewed, providing improvements for the sustainability of current and future transgenic insecticidal crops.

Temporal assortative mating of fall armyworm strains. Fall armyworm as a species contains two host strains that feed either on corn and sorghum (C-strain), or pasture and turfgrasses and rice (R-strain). These two strains, however, inhabit the same locations and are considered sympatric. ARS scientists in Gainesville, Florida, and Texas A&M University collaborators determined that there are significant behavioral differences between the populations. In trapping studies, male moths captured early in scotophase (before solar midnight) were 95 percent C-strain moths, while moths captured late in scotophase were 89.5 percent R-strain. This clear separation in behavior is evidence of assortative mating, which is likely a mechanism maintaining the separation of the two strains in sympatric habitats.

Residential citrus as a source of infective psyllids for commercial citrus groves. Citrus trees growing in residential areas are a source of Asian citrus psyllid that can fly to nearby commercial citrus groves and infect them with citrus greening disease. However, the level of psyllid movement between residential and commercial citrus trees is unknown. A 'Mark-Release-Recapture' was conducted by ARS researchers in Ft. Pierce, Florida, in which psyllids were marked with brightly colored fluorescent powders and released either in a neighborhood with many citrus trees or a commercial citrus grove adjacent to the neighborhood site. The psyllids were recaptured on yellow sticky card traps hung in both sites. By marking psyllids with

differently colored powders, the original release site and date of recaptured psyllids could be determined. A total of 15,300 marked psyllids were released during the study. Most of the marked psyllids recaptured in residential trees were from the neighborhood trees while only 15 percent were from the commercial grove. In contrast, approximately 50 percent of psyllids recovered in the grove were released in residential trees. About 40 percent of the psyllids changed habitats, but the change was skewed toward moving from the residential to commercial grove habitat. These results showed that there is a constant exchange of psyllids between the two habitats and that residential citrus trees are a source habitat of psyllids that emigrate into adjacent commercial groves. The data highlights the necessity of implementing effective psyllid management practices in residential habitats to reduce source populations of the psyllid.

Discovery of a potentially important source of biological control in potatoes. Potato growers in the Pacific Northwest have expressed interest in the use of commercially produced natural enemies for targeted release against aphid, mite, thrips, leafhopper, and psyllid pests. However, few if any natural enemy species which will attack the full suite of pests of concern to potato growers are available commercially. The whirligig mite (*Anystis*) was found to readily attack potato psyllid on weedy host plants of the insect pest's potato psyllid and was assayed to determine whether the mite also feeds on non-psyllid species which are pests of potatoes. ARS researchers in Wapato, Washington, and scientists at Washington State University collected mites from weedy hosts of the psyllid and assayed them to determine whether specimens harbored DNA of aphids, mites, thrips, or leafhoppers. All four pest taxa were detected in field-collected mites. Often a single specimen harbored DNA of multiple pest species, indicating that *Anystis* is a generalized and effective predator of arthropod pests common in potatoes. An insectary in Canada is now commercially rearing *Anystis* for purchase and release in Canada and has just recently expanded operations into Oregon, with aims to produce this mite in the United States for release in greenhouses, vineyards, and other agricultural crops. This research indicates that *Anystis* should also be considered for targeted use against pests that are important in potatoes.

Bacterial endosymbionts of potato psyllid. Insects often harbor bacterial endosymbionts that provide them with nutrition or protection from natural enemies, insecticides, pathogens, and plant defenses. The potato psyllid is a major insect pest of potato and vector of the pathogen that cause zebra chip, a devastating potato disease. ARS researchers in Wapato, Washington, and Washington State University scientists identified endosymbionts found in potato psyllid and compared them with endosymbionts found in two related non-pest psyllids, bindweed psyllid and atriplex psyllid. They found that bacterial communities were more similar in more closely related psyllids than in more distantly related psyllids and confirmed previous reports that both potato psyllid and bindweed psyllid harbor the pathogen that causes zebra chip disease of potato. They also discovered that potato psyllid harbors two distinct strains of the endosymbiont *Sodalis*, which in other insects has been found to provide nutritional benefits. Results of this study provide a foundation to conduct more in-depth research on interactions and co-evolution between psyllids and their bacterial endosymbionts.

Comprehensive description of herbicide non-target effects on key orchard predators. Minimizing pesticide use that harm natural enemies of orchard pests is a key component of conservation biological control in orchards. While the effects of insecticides on these natural enemies is well-known, there was virtually no information on the non-target effects of herbicides. ARS researchers in Wapato, Washington, and scientists at Washington State University characterized how seven herbicides affected predators by examining mortality, repellency, fecundity, movement, and predator efficacy on three species of spiders, two species of predatory mites, convergent ladybeetle, green lacewings, minute pirate bugs, and earwigs. Glufosinate, paraquat, and oxyfluorfen were consistently the most harmful pesticides to these predators. This information is being used by orchard consultants to improve spray programs to reduce harm to natural enemies and limit disruption of biocontrol efforts.

Bioactive peptides from SWD and thrips. Spotted-wing drosophila (SWD) and western flower thrips (WFT) are major global pests on small fruits and nursery crops, respectively. Insect neuropeptides (small protein molecules produced in the brain or nerve tissues) are potential targets for new insecticides because they are involved in most essential biological processes during life stages. ARS researchers in Corvallis, Oregon, identified seven short bioactive peptides from SWD and WFT using an insect cell-based in vitro assay. The binding activities of these peptides to their receptors were similar to their natural ligands, although injection or feeding of the bioactive peptides did not significantly affect the survival of the two insects. These results will be used to develop new insecticidal active ingredients to be used by nursery and small fruit growers to control both thrips and SWD.

Successful establishment, persistence, and impacts of introduced emerald ash borer parasitoids. The emerald ash borer (EAB) is a serious invasive forest pest that has devastated natural and urban ash forests and threatens the existence of North American ash species. Biocontrol programs against EAB began with the introduction of four insect parasitoid species from Northeast Asia (EAB's native range) between 2007 and 2015. ARS researchers in Gainesville, Florida, conducted a field study in the U.S. northeastern and north-central regions and found that three of the four introduced EAB parasitoids (*Spathius galinae*, *Tetrastichus planipennis*, and *Oobius agrili*) have successfully established persistent populations and significantly reduced EAB's densities, contributing to North American ash recovery and regeneration.

Upper thermal limits of pupae and pteromalid parasitoids. Determining upper thermal limits of *Rhagoletis indifferens* fruit fly pupae can have practical implications for disinfesting soils as well as for predicting differential how global warming will affect flies and their parasites. ARS researchers in Wapato, Washington, determined the upper thermal limits of western cherry fruit fly pupae and a wasp parasitoid inside fly puparia. They found that all fly pupae were killed at 49.4 degrees C and that the upper thermal limit of survival for fly pupae was 47.8 degrees C, while for wasps it was 51.1 degrees C. Information on heat kill of soil insects will be used by Washington State Department of Agriculture for developing guidelines to disinfest soils and organic waste of pest insects, which will reduce the spread of the pests via soils.

Water submersion kills western cherry fruit fly eggs and larvae in cherries. The Western cherry fruit fly is a quarantine pest of cherry in western North America. Determining the water tolerance of its maggots in cherries can help explain fly adaptations to stressful environments and can have practical value for sanitizing orchards. ARS researchers in Wapato, Washington, and APHIS researchers in Miami, Florida, determined the lethal effects of submerging infested cherries in water for 4, 8, and 12 days on fly eggs and maggots. They observed that 8- and 12-day water submersions killed 100 percent of eggs and maggots. Results are being used as a basis for recommendations by APHIS for orchard sanitation options for managing fruit flies.

Bacterial endosymbionts of leafhopper pests discovered. Many insects harbor bacteria called symbionts that provide them with nutrition, or that protect them from natural enemies, insecticides, and plant defenses. It remains largely unknown how these bacterial symbionts alter the ability of insect vectors to spread crop pathogens. ARS researchers in Wapato, Washington, and Kimberly, Idaho, and scientists from Washington State University, identified symbionts from leafhopper vectors of phytoplasma pathogens that cause plant diseases including cherry x-disease (a.k.a, little cherry disease). They discovered several previously unknown symbionts that may be beneficial to the vectors and uncovered new interactions between symbionts and the phytoplasma pathogens. Notably, the presence of the symbiont *Wolbachia* was associated with a higher likelihood that leafhoppers also carried phytoplasmas. This information will be used by scientists to conduct more in-depth research on leafhopper symbionts and how they influence biology and management of leafhopper vectors of cherry x-disease.

Using eDNA technology to find invasive species in green yard waste. As large cities begin to overrun their landfill capacities, they are starting to look for alternative locations to handle the waste stream. Seeing an opportunity to bring in revenue, rural communities offer to handle municipal waste in their landfills. Unfortunately, many rural communities are also locations of agricultural production, which are vulnerable to attacks by invasive species. ARS researchers in Wapato, Washington, used a combination of ecological niche modeling and eDNA sequencing to determine whether green yard waste could be a pathway for invasive species to enter and become established in an agricultural community with a landfill. They found the green yard waste contained DNA from several quarantine-actionable pests that pose a threat to agriculture in Washington State. Reports of research results and positive identifications of potential invasive species were made to federal and state regulatory agencies to aid in risk management decision making related to the potential for the transport of invasive species through municipal waste relocation programs.

The phylogeny and population biology of fall armyworm populations. The threat of invasive species is increasing with the expansion of global trade and habitat disruption. For example, fall armyworm, a noctuid moth native to the Americas, has recently become established in most of the Eastern Hemisphere and projections suggest this could result in significant economic losses on a global scale. The species has traditionally been subdivided into two populations that differ in their propensity to use different plant hosts, which is significant for determining potential crop risks, but inconsistencies in the genetic and phenotypic descriptions of these “host strains” has led to controversy about their composition and even existence. ARS scientists in Gainesville, Florida, used phylogenetic analysis to uncover genetic differentiation between populations that further supports the existence of the host strains and provided evidence that they are subject to different selection pressures. Genetic comparisons of moths collected from multiple locations revealed significant differences in the presence of different molecular markers that also indicate there are regional variations in host strain behavior. The data confirm that the host strains are a fundamental characteristic of the species that needs to be taken into consideration when developing monitoring and mitigation strategies.

Using fungi to control the red palm weevil. The most devastating pest of palm trees globally is the red palm weevil, in part because infested trees can harbor weevils undetected until trees are dying. Control generally relies on synthetic insecticides that are not sustainable, negatively impacting both the environment and biological diversity. ARS scientists in Gainesville, Florida, led an international collaboration to use acoustic monitoring to track the decline of insect activity inside trees after various treatments. Acoustic bursts from weevils in date palm plantations were reduced within two to three months after treatment with entomopathogenic nematodes and fungi, aluminum phosphide fumigation, and insecticidal spray treatments. The insecticides and entomopathogenic treatments provided the most effective treatments in heavily infested plantations. This demonstrates the utility of acoustic monitoring in assessing the effectiveness of weevil control, as well as demonstrating the efficacy of new and ecologically friendly management tools for this devastating pest.

Predatory ladybird beetles’ diversity and their prey whitefly species in Hawaii. Ladybird beetles (LB) are an important group of insect predators that prey upon many pests, including aphids, scale insects, mealybugs, and whiteflies, in crop fields, orchards, gardens, urban landscapes, and greenhouses. Several LB species have been purposefully and inadvertently introduced into the Hawaiian Archipelago for more than 100 years. Some have a tremendous potential to be used in the biological control of invasive pests, including whiteflies, but studying their predatory potential would not be possible without documenting the species of LB and their prey. ARS scientists in Ft. Pierce, Florida, updated the list of LB in Hawaii with 2 new state records, 17 new island records associated with 13 species, and various prey records, including 5 whitefly species. The update includes new geographic distribution records from the eight main and

Northwestern Islands. The new records demonstrate the value of ongoing sampling and regular examination of undetermined collections material. These findings will assist stakeholders who work with insect management in Hawaii, including pest management practitioners, agriculturalists, conservationists, and biodiversity specialists.

Elevated stress responses contribute to feeding habits of western corn rootworm (WCR) larvae. WCR larvae feed on the roots of a narrow range of grasses, including maize and the cellulosic biofuel crop *Miscanthus*, and this damage results in significant losses in U.S. yields. Farmers manage WCR damage by growing maize hybrids that express one or more insecticidal *Bacillus thuringiensis* proteins, applying chemical insecticides, or by rotating crops grown annually in the same field to include plants that are not viable hosts for WCR larvae. Each of these management practices are threatened by development of resistance. ARS researchers in Ames, Iowa, and an international team of collaborators investigated the changes in gene expression among WCR larvae that fed on roots of maize, *Miscanthus* and switchgrass, and sorghum (non-host), compared to a control group. The fewest number of significant changes in gene expression were observed for larvae that fed on maize, suggesting many of the same genes are involved in adaptation to these host plants. The greater number of differentially expressed genes between maize and other host plants, including the switchgrass biofuel crop, were predicted to function mostly in cell stress response mechanisms. Elevated stress response was interpreted to indicate WCR larvae are less-well adapted for feeding on non-maize and non-*Miscanthus* crops. This information will help identify target genes for improved host plant resistance breeding in maize, and guide other research of university, government, and industry scientists for evaluating and improving WCR management on maize and other cellulosic biofuel crops.

Abundance, ecology, and distribution of *Rhagoletis* flies in central Washington. Pest and non-pest fruit flies trapped in surveys can provide new information on regional fly abundance. ARS researchers in Wapato, Washington, and scientists at the Washington State Department of Agriculture in Yakima, Washington, analyzed data from surveys for pest cherry fruit flies and apple maggot flies in central Washington to determine the relative abundance of fly species and factors related to their abundance. They found that cherry fruit flies were common, apple maggot flies were very rare, and that walnut husk fly was the most abundant fly species. Abundance data suggests apple maggot is less tolerant of arid central Washington summers than other fruit flies and that relative fly abundance depend on site, tree type, and seasonal period. Results can be used by tree fruit industry representatives and APHIS to show that pest apple maggot flies are unlikely to develop large populations in apple-growing regions. This will help in developing Washington State Department of Agriculture guidelines on pest level tolerance, designation of areas as low pest prevalence, how the pest should be managed, and APHIS negotiations with export markets on movement of apples from these regions.

Adaptation of apple maggot fly on large-thorn hawthorn in Washington. Apple maggot fly is a quarantine pest of apple in western North America. If a fly population has adapted to non-apple host plants fly management can be affected because managing the fly in apple alone would be insufficient to reduce the threat of flies attacking apples. ARS researchers in Wapato, Washington, collaborated with scientists at the Washington State Department of Agriculture and the University of Notre Dame to determine if flies attacking large-thorn hawthorn in north-central Washington have adapted to the plant. They found evidence that the flies have adapted to large-thorn hawthorn; flies from large-thorn hawthorn emerged earlier than flies attacking apple and the flies preferred to attack and lay eggs in the hawthorn instead of apple. This finding has resulted in new quarantine boundaries around affected orchards and active control measures by the county pest board targeting the flies, all of which will help growers export their apples to foreign markets.

Infestation of a native honeysuckle by a native fruit fly. Host plant shifts have been hypothesized as an important factor in initiating population divergence and speciation in fruit flies in the genus *Rhagoletis*. The apple maggot fly is a model organism for host race formation via host shifts, but its sibling species snowberry maggot fly, which is hosted by snowberry only, has not been implicated as having shifted host plants since its evolution from apple maggot ancestors. ARS researchers in Wapato, Washington, and scientists at the Washington State Department of Agriculture and the University of Notre Dame determined that snowberry maggot also infests orange honeysuckle in Washington State. This represents the first reported case of a native honeysuckle infested by a native *Rhagoletis* in North America. This finding helps the academic and scientific community better understand the evolution of host plant switching by fruit flies and highlights that state agricultural officials must be constantly prepared for fly species shifting from wild host plants to economically important plants.

Erythritol formulations have minimal impacts on beneficial insects. Spotted wing drosophila (SWD) is an economic pest of small fruits and cherries. A food-grade insecticide such as erythritol is a sustainable way to manage SWD provided there are minimal non-target impacts. Honeybee adults were not impacted by direct feeding and would typically not forage on crops as erythritol is sprayed post-bloom, but the impact on immature bees was unknown if some adults carried this back to the hive. ARS researchers in Corvallis, Oregon, found that erythritol formulations introduced to honeybee brood had no discernible impact on survival. Also, feeding on erythritol by a SWD parasitoid had minimal impacts on survival, especially since wasps prefer to feed on other sugar sources such as floral nectar. As minimal impacts on honeybees have been observed, companies can consider registering erythritol in new alternative formulations. This technology will improve SWD control and reduce chemical insecticide use by fruit growers.

CRISPR/Cas9 gene editing feasible in *Lygus*. CRISPR/Cas9-mediated gene editing is the foremost tool available for functional genomics approaches and targeted genomic engineering applications; however, the technique is species-specific and often requires significant optimization, especially in non-model organisms such as *Lygus hesperus*. The applicability of the CRISPR/Cas9 system for this species was successfully demonstrated by a team of ARS researchers in Maricopa, Arizona. Eggs injected with the Cas9 enzyme and sgRNAs targeting either cardinal or cinnabar, two eye pigmentation genes, developed red eyes instead of brown eyes typical of wildtypes. Although atypical coloration in both experimental groups largely persisted throughout the nymphal stages, adult manifestation of the phenotype was limited to the cinnabar group, and atypical eye coloration was associated with disruptions in the sequence of the respective target genes. The heritability and persistence of the red eye phenotype across multiple generations demonstrated that CRISPR/Cas9 gene editing can be applied to *L. hesperus* and that eye pigmentation genes are useful for tracking genetic manipulation.

A push-and-pull strategy for spotted wing drosophila pest management. Push-pull strategies use attractants and repellents to manage pests and effective push-pull systems can divert pests away from crops and control them without broad-spectrum pesticides. ARS scientists in Beltsville, Maryland, continued collaboration with scientists in Towson University, Rutgers University, and the University of Maryland-Eastern Shore to develop a push-and-pull strategy utilizing an optimized controlled-release dispenser for spotted wing drosophila (SWD) attraction and powerful SWD repellents to reduce SWD populations and infestation in blueberry orchards. Because the natural semiochemicals were used in a push-and-pull strategy, it has provided growers/farmers an efficient, convenient, safe, and environmentally friendly alternative for SWD control.

Tolerance of a ladybug predator to several insecticides. Insecticides are commonly used as a pest management strategy against pests in Brassica crops such as collard and kale. Collaborative research between researchers in Brazil and ARS researchers in Charleston, South Carolina, assessed 10 commonly

used insecticides for their compatibility to a ladybug predator (*Eriopis connexa*) in Brassica crops. Although feeding behavior by the beetle was affected in some cases, several of the evaluated insecticides were compatible predator ladybug survival and egg laying. The results of this research demonstrate that this natural enemy can have a positive impact on managing pests in the Brassica cropping system when several selected insecticides are used by growers.

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

Asian citrus psyllid control using ethyl formate. Asian citrus psyllid (ACP) is a devastating pest of citrus, as it spreads the disease Huanglongbing that curtails fruit yield and quality. Southern California is impacted by ACP quarantines, but the packing and juicing operations in California’s San Joaquin Valley are not. ARS scientists in Parlier, California, developed a postharvest fumigation treatment with ethyl formate to control ACP after fruit is binned and loaded on a trailer and before it is transported from the grove. This innovative treatment limits ACP spread and reduces broad-spectrum insecticide use. ARS scientists also developed an ethyl formate treatment that will begin replacing methyl bromide treatments of table grapes imported from Chile, which represents the greatest U.S. usage of this fumigant on fresh fruit. Data generated by this research was submitted to the U.S. Environmental Protection Agency and California Department of Pesticide Regulation to support Section 3, Federal Insecticide, Fungicide, and Rodenticide Act registration.

Repellents for pest ambrosia beetles. Redbay ambrosia beetle and tea shot-hole borer are vectors of the fungal diseases laurel wilt and *Fusarium dieback*, which infect avocado, woody ornamentals, and native forest trees. Incorporating a repellent into pest management programs may reduce the incidence of these diseases. ARS scientists in Miami, Florida, identified piperitone as a new beetle repellent and compared its efficacy to two other repellents, verbenone and α -farnesene. Beetle captures in traps baited with lures were compared to those containing lures plus a repellent (a push-pull design). Results showed that farnesene was ineffective; however, piperitone and verbenone were equally effective, reducing captures by 50-70 percent for 10-12 weeks. Since piperitone is less expensive than verbenone, it has become the standard beetle repellent. This study identifies an economical push-pull strategy avocado growers can use to manage the two beetle vectors.

Controlling invasive and quarantine horticultural pests. Brown marmorated stink bug (BMSB) is an invasive insect pest that damages fruit, vegetable, and ornamental crops. BMSB adults overwinter in container consignments and vehicles, many of which are exported to countries requiring phytosanitary treatments to control pests. In 2022, ARS scientists in Parlier, California, were asked to develop a treatment that could be applied to exports when they arrive in Australia and New Zealand. An ethyl formate fumigation protocol was developed to control BMSB, which allowed U.S. manufacturers to keep exporting automobiles to Australia and New Zealand—a projected annual value of \$10 billion.

Using DNA metabarcoding to assess insect diversity in citrus orchards. Citrus hosts both beneficial and pest insect species, but species identification is difficult and large-scale insect diversity assessments are challenged by cryptic morphology and a lack of taxonomic experts. Scientists in the Sino-American Biological Control Laboratory, an ARS-associated lab in Beijing, China, analyzed the composition of insect communities in citrus orchards using DNA metabarcoding followed by data analysis on two cloud databases and analytical platforms—the Barcode of Life Data System (BOLD) and Multiplex Barcode Research and Visualization Environment (mBRAVE). The species identified using BOLD were then searched in citrus pest databases and the literature to designate their classification as a pest, parasitoid, predator, or pollinator. These results provide a valuable resource for research on citrus pest management and beneficial insect exploration.

Navel orangeworm response to synthetic pheromone lures. Navel orangeworm is a serious insect pest of almonds, pistachios, and walnuts and is commonly managed with mating disruption technique. Optimizing control technologies based on pheromone mating disruption requires determining how biological clocks and external temperatures affect the timing of sexual activity of moth pests like the navel orangeworm. ARS researchers in Parlier, California, and collaborators from California State University, Fresno, and University of California, Riverside, gathered and analyzed two years of camera trap data to build on information from previous studies based only on field observations of males attracted by laboratory-reared females. They found that in summer, males exhibited the greatest sexual activity in the last few hours before sunrise and began mating earlier in cooler times of the year. They also found that, unlike previous reports, peak capture times changed with season but not with transient temperature changes. Improved understanding of day-night male activity patterns will improve data monitoring and interpretation and enable optimizing navel orangeworm mating disruption. These findings could improve navel orangeworm controls and reduce insecticide use in almonds and pistachios planted on approximately 1.5 million acres of land with an annual farm gate value of nearly \$8.5 billion.

Identifying pistachio hull factors associated with insect damage. The navel orangeworm (NOW) is the primary insect pest of pistachios, a specialty crop worth more than \$2.5 billion in California. NOW controls costs more than \$50 million annually and more information is needed about NOW biology and interaction with its pistachio host to reduce damage by identifying high risk orchards. ARS researchers in Parlier, California, conducted an 11-year study using processor grade sheet information to identify nut factors associated with increased insect damage. Two nut factors related to early hull split, percent adhering hull and dark staining, were strongly correlated with damage. An improved grower understanding of the management practices that cause early hull split, including irrigation practices, will reduce the frequency of its occurrence, thereby reducing insect damage and increasing nut quality.

Sulfur dioxide for controlling navel orangeworm on stored pistachios. Navel orangeworm (NOW) is a major pest of stored tree nuts, including pistachios in California. ARS scientists in Salinas, California, evaluated sulfur dioxide (SO₂) fumigation as an alternative treatment for postharvest control of NOW on stored pistachios. They found that three-hour fumigations were effective against NOW at different life stages. Complete controls of eggs, larvae, and pupae were achieved in three-hour fumigations with 0.2, 2.0, and 1.0 percent SO₂, respectively, and large-scale three-hour fumigations of pistachios with 1.6-1.8 percent SO₂ had complete control of 6th instar larvae in infested pistachios. The results showed that SO₂ fumigation has potential to control NOW on stored pistachios as well as to control other postharvest pests on stored products.

Compost suppresses a common soilborne lettuce pathogen. Soilborne plant pathogens are a significant constraint on California lettuce production valued at \$2 billion dollars annually. Soil fumigation is too expensive for lettuce growers and is incompatible with organic production standards. ARS researchers in Salinas, California, showed that adding compost to soil reduces the abundance of the soilborne lettuce pathogen *Fusarium oxysporum*. Results from this research provide growers with methods to reduce a common soilborne plant pathogen of lettuce.

Identification of host kairomones for lychee erinose mite. The invasive lychee erinose mite (LEM) is a serious pest of lychee fruit trees in Florida. This tiny mite lives mainly on the underside of the leaves, preventing flowering and fruit production. Little is known about the chemical ecology of LEM and no known attractants or repellents exist. ARS scientists in Miami, Florida, and University of Florida researchers investigated potential host-based attractants or host defensive compounds induced by LEM infestation. Qualitative comparison uninfested and infested lychee plants revealed that six volatile organic compounds (VOCs) were present in almost all samples of uninfested and infested lychee plants. Behavioral

(olfactometer) bioassays demonstrated that the chemical concentrations of these six VOCs significantly influenced LEM attraction, which was typically most attractive at lower concentrations. The findings of this study provide useful information on the development of a field lure that can be used in LEM pest management programs.

Resources for flighted spongy moth surveillance. Trees and forests in the northeastern United States suffer tremendous damage from outbreaks of the spongy moth (formerly known as gypsy moth), a defoliating pest. The spongy moth subspecies present in the United States is characterized by flightless female moths. However, two Asian subspecies, collectively known as the flighted spongy moth, pose a significant invasive threat and are expected to have a much wider distribution in the United States due to its flightworthy females. Identifying the genetic differences between spongy moth and flighted spongy moth will assist in surveillance and identification of invading flighted spongy moth populations. ARS researchers in Beltsville, Maryland, and Beijing Forestry University collaborators assembled and compared gene expression profiles of two U.S. populations of spongy moth and two Chinese populations of flighted spongy moth and identified distinct differences in gene expression between the U.S. and Chinese populations. All four populations contained surprisingly high loads of a persistent RNA virus, with variant viral sequences that were associated with specific host populations. The population-specific virus and moth sequence data generated during this research are available at the NCBI National Center for Biotechnology Information Genbank and Sequence Read Archive databases and can be mined for genetic markers to be used in flighted spongy moth surveillance.

New plant-based toxicants against the Caribbean fruit fly identified. Tephritid fruit flies are among the most serious agricultural pests worldwide. Current management typically relies on bait sprays or bait stations that incorporate insecticides, but concerns have been raised regarding the adverse effects on the environment and the development of increased pest resistance. ARS researchers in Miami, Florida, evaluated plant essential oils extracted from three species of chamomile plants, commonly used in therapeutic applications: German chamomile, Roman chamomile, and Chinese chamomile. Results showed that Chinese chamomile essential oil demonstrated the most significant insecticidal activity against Caribbean fruit fly female adults. This study discovered that Chinese chamomile essential oil is a promising source of potential alternative pesticides for Caribbean fruit fly.

Novel toxicants against the Caribbean fruit fly. Natural products with insecticidal effects against insect pests have the potential for use as alternatives to synthetic insecticides. Phthalimides (isoindoline-1,3-diones, and their N-substituted analogs) are found in natural products, can be synthesized in the laboratory, and have demonstrated biological activities including insecticidal attributes. In collaboration with Marmara University (Turkey), ARS scientists in Miami, Florida, investigated 13 phthalimide derivatives for their toxicities against female Caribbean fruit fly. Results showed that three phthalimide derivatives exhibited potent insecticidal activity against Caribbean fruit fly. The physicochemical properties of these phthalimides demonstrated that higher lipophilicity tended to show good insecticidal activity. This study revealed key structural features responsible for the insecticidal activity and can be promising for the development of new biopesticides.

Vibrational communication identified in the spotted lanternfly. The spotted lanternfly is a polyphagous insect pest that invaded the United States in 2014 and has spread from Pennsylvania to several northeastern states, threatening the agriculture production of many crops. ARS scientists in Miami, Florida, and APHIS collaborators demonstrated that adult and fourth instar spotted lanternfly are attracted to broadcasts of 60-Hz acoustic and vibrational stimuli in the laboratory. This finding expanded current information about frequencies that are attractive to spotted lanternfly and suggests that a vibroacoustic trap may be developed for management of spotted lanternfly in the field.

Enhancing trapping to detect and monitor adult wireworms. The corn wireworm is an economically important pest that feeds on root and tuber crops. A recent discovery of the corn wireworm sex attractant by ARS researchers in Charleston, South Carolina, and collaborators provides a new method to detect and monitor this pest during its adult stage. A collaborative study among researchers at North Carolina State University, University of California, Virginia Polytechnic Institution & State University, University of Florida, and ARS researchers in Charleston, South Carolina, optimized trap type and placement for corn wireworm capture and monitoring and found that the population of the corn wireworm varies in the U.S. southeast, and that traps placed at waist-high and with lures of up to two-weeks-old resulted in the most capture of beetles. Findings from this research will help researchers develop practical pest management options for wireworms.

Fungal effects on morphological changes of insects. Biological control using the fungus *M. anisopliae* has shown potential for managing various insect pests, including tephritid fruit flies. Destruxin A (DA) is a mycotoxin isolated from *M. anisopliae*, but the mechanism of toxicity against insects remains unknown. Scientists from Guangdong Academy of Agricultural Science in China and ARS in Miami, Florida, used histopathological methods to investigate the effect of DA on target cells and tissues of the silkworm *Bombyx mori*. At low doses, the hemocytes were the most sensitive to DA and at higher doses, the muscle cells, fat body, and Malpighian tubules showed morphological changes 24 hours after treatment. This study elucidated the target sites in *B. mori* that responded to DA treatment, indicating that the defensive hemocytes were the first cells damaged. The results of this study will help develop mycopesticides and novel immunosuppressants for improved management of tephritid fruit fly pupae.

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).