Agricultural Research Service • National Program 304 • Crop Protection and Quarantine

FY 2015 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, there are 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.

The current Action Plan (2010-2015) can be viewed online at http://www.ars.usda.gov/research/programs/programs.htm?np_code=304&docid=24769.

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: Protection of Agricultural and Horticultural Crops:** improving existing and/or developing new, innovative control strategies for pests in traditional and organic agricultural and horticultural systems
- **Component 3: Protection of Natural Ecosystems:** 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 and Quarantine:** 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 2015. The results are presented under the components and problem statements of this program's 2010-2015 Action Plan. The report below is not intended to be a progress report describing all research conducted during the 2015 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 co-leaders of National Program 304, Kevin Hackett (Kevin.Hackett@ars.usda.gov) and Rosalind James (Rosalind.James@ars.usda.gov).

Component 1 – Systematics and Identification

Russian wheat aphid genome sequenced. More than 5,000 aphid species exist and more than 100 are economically important crop pests. Previously, the sole genomic model available for understanding aphid-plant interactions was the pea aphid. Although the pea aphid and Russian wheat aphid share many taxonomic and biological traits, they differ in how they feed on the plant and the plant damage they cause. Therefore, a multi-institutional, international, collaborative effort was organized and led by ARS scientists in Stillwater, Oklahoma, to sequence the genome of the Russian wheat aphid. This effort resulted in a new aphid genome assembly (called Dnoxia_1.0) that is now available to the public through GenBank. This research provides a new comparative model for understanding aphid-plant interactions because the Russian wheat aphid produces phytotoxins when it feeds, and the pea aphid does not. This genome also made it possible to develop RNA interference technology for aphid control and will be important to future pest management technologies.

ARS provides valuable mite and insect identification services. ARS entomologists in Beltsville, Maryland, provide prompt and urgent information and identifications to APHIS, the State Department, and U.S. universities for important invasive and adventive mites and insects. In FY 2015, the unit identified 21,733 insect and mite specimens (approximately half were urgent identifications, and most of the rest were needed promptly). These identifications are of paramount importance to trade issues, affecting regulation and quarantine decision-making to protect U.S. agriculture from invasive pests.

Developed new tools for unambiguous identification of pest fruit flies. ARS researchers revealed morphological characters that distinguish species of *Anastrepha*, the largest and most economically important group of fruit flies in the American tropics. Twenty-eight previously unknown species (including species that attack guava and annonas) were named, described, and illustrated; these species and additional character data were included in further development of an electronic identification tool for the nearly 300 species in this group. Numerous new DNA samples were obtained for analysis and new sequences for the gene ITS1 were analyzed to resolve the South American fruit fly complex. This work will be useful to scientists and regulatory agencies involved in management and control of pest fruit flies.

First record of an Asian natural enemy of stink bug in the United States. The invasive brown marmorated stink bug was identified in the United States in 2001, has spread to the west coast, and been found in 41 states. Thirteen states, on both coasts, have reported significant agricultural damage, in addition to the nuisance of the stink bug as a building invader during the late fall and winter. In its native Asian range the stink bug is a sporadic, regionally localized pest. In its introduced range in the United States it feeds on a wide variety of fruit and vegetable crops and has become a significant agricultural pest. Chemical control strategies have not prevented it from causing economic damage. In Asia the stink bug's natural enemies help prevent it from reaching damaging numbers. These natural enemies were brought to quarantine laboratories in the United States for evaluation by ARS and state researchers as potential biological control agents. In 2014, an ARS survey of resident natural enemies revealed that a parasitic wasp, which is a key natural enemy of brown marmorated stink bug in Asia, was found at a Maryland survey site. It is not known how the wasp arrived at that site, but it is presumed as an accidental hitchhiker during trade. If it survives and spreads to other regions, it will help to reduce populations of the brown marmorated stink bug in North America.

Completed a monographic study of the *Renodaeus* group of species, adding 28 new species. Eight genera (including four new to science) in the *Renodaeus* group of species in the plant bug subfamily Ceratocapsinae were revised to accommodate 48 species, including 28 species new to science. Photographs of the adult males and females, illustrations of male genitalia, and keys to the genera and species were provided to help distinguish these bugs. Little is known of the biology of these plant bugs, but most are thought to be predators of aphids, scales, lepidopteran larvae and eggs, and other small arthropods. This work will allow researchers to identify and investigate the potential of these bugs for biological control.

Optimizing control strategies with genetic identification in the invasive *Scirtothrips dorsalis* species complex. It is difficult to identify invasive arthropods; the task is particularly difficult if multiple species are morphologically indistinguishable. Fortunately, direct analysis of their genetic code can be used to accurately identify them. Species identification is a fundamental requirement for plant quarantine and biological control programs, as well as effective integrated pest management strategies. The chilli thrips (*Scirtothrips dorsalis*) is a plant pest of many ornamental, food, and fiber crops with a rapidly expanding global distribution. What was thought to be a single species, based on appearance, has been found to be many species that have different behaviors that could require different control strategies. ARS scientists in Fort Pierce, Florida, collaborated with the Universities of Florida and California to develop a comprehensive genetic identification strategy for these cryptic species. Researchers have shown that nine of the eleven species the scientists observed can only be differentiated using this genetic strategy. One member of the complex is highly invasive, feeds on multiple host plants, and is likely the species that transmits the virus. Two other species also feed on many plant species and appear to be at an earlier stage of invasion. Other members of the complex only occur in their native range and vary with respect to pest status and diet. When invasive pests such as the chilli thrips enter the United States, rapid and accurate taxonomic characterization of the invader, as well as a determination of its position in context of global diversity, will allow rapid implementation of effective control strategies.

Two new bacterial species for safe microbial control of insect pests. ARS researchers in Beltsville, Maryland, discovered two new insecticidal *Chromobacterium* species. One species was found to be toxic to caterpillar pests such as the diamondback moth and gypsy moth, but not to beetle pests such as seedcorn maggot or red flour beetle. In contrast, the other species exhibited pronounced toxicity to the seedcorn maggot, but less activity against diamondback moth. ARS researchers found that at least two distinct insecticidal factors are produced by the two new species. Commercial development will result in a wider range of options for safe microbial control of insect pests, thereby benefitting farmers, pest managers, and consumers.

Component 2 – Protection of Agricultural and Horticultural Crops

RNA interference technology for control of aphid pests of crops. Cereal aphids such as greenbugs and Russian wheat aphids are the primary pests of wheat and infestations can require yearly application of insecticides. The development of aphid-resistant wheat lines has been the most economical solution for control. However, aphid biotypes have developed in both greenbug and Russian wheat aphids that overcome wheat resistance. Therefore, new technologies are needed that are not specific to any given aphid biotype. Double-stranded RNA (dsRNA) constructs that are complementary to specific aphid genes can prevent those genes from functioning and kill the insect. ARS scientists developed two dsRNA constructs that have proven to be highly effective in causing Russian wheat aphid mortality in laboratory tests; this novel technology offers a new approach in breeding wheat plants with highly specific resistance to aphids. A U.S. patent has been filed on these two constructs.

Plant virus-based expression vector developed for RNAi delivery. Double-stranded RNA (dsRNA) methods hold good potential for developing a wide variety of pest-resistant crop varieties and this resistance can be very specific toward target pests. However, the technology for introducing dsRNA into plants is not well developed, thereby limiting its current use. ARS scientists in Fort Pierce, Florida, demonstrated the effectiveness of using a plant virus-based expression vector as a method to deliver dsRNA into plants. In collaboration with Brazilian scientists, the ARS scientists also identified dsRNA molecules that are effective at inducing a lethal RNA interference response in a broad group of insects including whiteflies and psyllids. This delivery technique was successfully demonstrated for whiteflies.

Transgenic (Bt) crops do not negatively affect important beneficial arthropod parasitoids and predators. Globally, millions of hectares of Bt transgenic crops are cultivated, but concerns remain in the United States about the risks they may pose to nontarget and/or beneficial organisms such as arthropods that are natural enemies of crop pests and that supply important ecological services. ARS scientists in Maricopa, Arizona, and Ames, Iowa, with researchers at Cornell University and Agroscope in Zurich, Switzerland, showed that over multiple generations the development and reproduction of a parasitic wasp, a predatory beetle, three predatory true bugs, a predatory mite, and a predatory green lacewing were not affected by ingestion of prey that had fed on Bt plants/crops. The natural enemy species that were tested represent some of the most abundant and important species in cropping systems. The prey used were either resistant to or not susceptible to the Bt proteins in the crop plants. This approach eliminates prey quality issues, allowing the direct effects of Bt proteins to be accurately assessed under realistic exposure scenarios. Results from these studies may be valuable to governmental authorities who are responsible for regulating transgenic crops, scientists who are concerned with developing integrated pest management strategies for pest control, and the general public, which is concerned with the environmental effects of biotechnology.

Characterization of codling moth nicotinic acetylcholine receptors targeted by insecticides. Codling moth, the major insect pest of apple, is controlled with a combination of pheromonal mating disruption and multiple applications of chemical insecticides. There is concern that the multiplicity of chemical insecticides may have negative effects on beneficial insects. Two classes of insecticides used in codling moth control programs are neonicotinoids and spinosads, each of which target nicotinic acetylcholine receptors present in the insect nervous system. ARS researchers in Wapato, Washington, identified and characterized the expression of gene transcripts encoding 12 different codling moth nicotinic acetylcholine receptor subunits. Comparison of the codling moth nicotinic acetylcholine receptor subunits with those expressed in the honey bee, which is sensitive to neonicotinoids, revealed the presence of two codling moth proteins that are not present in the bee. This information could lead to the development of novel insecticides that specifically control codling moth without having negative effects on honey bees.

Release of stink bugs from competition with Bt-controlled lepidopteran pests contributes to their increased presence and damage to Bt-cotton. Reduced insecticide use has been the dominant hypothesis (in the literature) to explain stink bug outbreaks associated with cotton genetically engineered (GE) to control lepidopteran insect pests, but release from competition from these lepidopterans (pests controlled by the cotton) may also contribute to stinkbug outbreaks, i.e., while GE cotton's target pest is well-controlled, secondary pests, like stink bugs, fill in the gaps the target pest has left behind. ARS researchers in Tifton, Georgia, and university collaborators determined that two stink bug species, *Euschistus servus* and *Nezara viridula*, strongly preferred laying their eggs on undamaged cotton plants, rather than caterpillar, *Helicoverpa zea* and *Heliothis virescens*, damaged (fed-upon) plants, and *E. servus* left *H. zea* damaged plants at a higher rate. These and previous results of resource competition between *E. servus* and *H. zea* demonstrate that release from competition likely contributed to stink bug outbreaks in cotton. The researchers also determined that, because of effective suppression of lepidopteran herbivorous cotton pests, GE cotton produces less of its natural defensive compounds, making the plant less toxic to attacking (chewing) pests, resulting in an increase in the density and reproduction of another cotton pest, the cotton aphid. Identification of these mechanisms underlying these interactions increases ARS scientists' understanding of how insect-resistant crops impact herbivore communities and helps underpin the sustainable use of GE varieties.

Information on the physiologic effects of migratory flight in the rice leaf roller moth will help predict pest outbreaks. In a cooperative study between ARS researchers in Ames, Iowa, and China, flight mills were used to examine the reproductive consequences induced by different flight scenarios on rice leaf roller moths, a severe migratory pest of rice in Asia. ARS researchers found that the influences of flight on reproduction depend on the age at which the moth begins migration, flight duration, and consecutive nights of flight. Flight synchronizes egg-laying in groups of migratory females, which may promote high-density larval outbreaks; a strategy some migratory crop pests in North America may also use. This information will be used to better understand the mechanisms for controlling the explosive growth in the outbreaks of insect populations, thus leading to better methods for their prediction and management.

Management strategies for zebra chip of potato based on psyllid biology, behavior, and host range. Zebra chip (ZC) is a devastating disease of potato affecting growers in the United States, Mexico, Central America, and New Zealand. ZC is caused by the bacterium Liberibacter, which is rapidly transmitted to potato plants by the potato psyllid, its insect vector. Since disease resistance to ZC is not yet available in commercial potatoes, an integrated approach based on psyllid biology, and host range is needed to manage this disease and minimize losses. ARS researchers in Wapato, Washington, determined that potato psyllids can transmit the bacterium to potato plants in as short as four minutes. In collaboration with scientists at DuPont Crop Protection Company in Delaware, ARS scientists assessed the use of cyantraniliprole, a new and safer insecticide that has proven to be effective at deterring feeding behavior of potato psyllid and preventing transmission of the ZC pathogen by the psyllid. Infected seed potatoes were found to either not germinate or to produce a few, short-lived plants, showing that seed potatoes are not an important source of ZC disease, especially in areas where the potato psyllid vector is absent. Alternative host plants have a critical role in the psyllid's ecology, as sources of ZC in weeds and overwintering hosts. ARS scientists determined that potato psyllid successfully develops on a number of plants in the morning glory family, including several varieties of ornamental morning glory, field bindweed, and sweet potato. Matrimony vine, a perennial potato relative and common weed in the Pacific Northwest was also determined to be an important host of potato psyllid throughout winter months in Washington, Idaho, and Oregon, and constitutes a major source of psyllids colonizing Pacific Northwest potato crops. This research provides valuable information needed to develop effective management tools for this important disease and underscores the challenges potato growers face.

Glyphosate impacts growth of shoots from underground vegetative buds of the noxious perennial weed leafy spurge. ARS scientists in Fargo, North Dakota, discovered that sublethal rates of foliar-applied glyphosate result in an uncontrolled proliferation and atypical appearance of shoots from underground vegetative buds. Researchers then exploited glyphosate's growth regulating properties to investigate metabolic processes involved in this phenomenon. Glyphosate was found to induce complex interactions involving the shoot growing point, plant hormone biosynthesis and signaling, cellular transport, and detoxification mechanisms. These results suggest that sublethal levels of glyphosate affect the control of genes involved in gibberellic acid and ethylene biosynthesis, and auxin transport.

Novel iron-binding metabolites and the genetic mechanism responsible for their production discovered for *Metarhizium robertsii* (MR), an important insect-pathogenic fungus and biopesticide. The low solubility of iron in nature requires many microbes to produce small molecule metabolites that are secreted into the environment where they bind iron and then are recovered and used by the microbes. These molecules are weapons deployed by invading pathogens to control the limited iron resources of the hosts, which themselves have evolved defense mechanisms to protect their iron from the invading pathogen. ARS scientists in Ithaca, New York, determined the chemical structure of novel iron-binding compounds and the genes that regulate their biosynthesis from *Metarhizium robertsii* an important insect-pathogenic fungus and biopesticide. These molecules have unique chemical features, including the presence of two sugar units that distinguish them from all other iron-scavenging molecules produced by fungi. Inactivation of the genes controlling the biosynthesis of one of these iron-binding compounds reduced *M. robertsii* virulence 2-3 fold. These compounds contribute to the ability of this fungus to successfully infect and kill insects and identified genes could be manipulated to enhance the performance of this fungus as a biopesticide.

Breakdown products of plant odors attract the Asian citrus psyllid and could be the basis for an attract-and-kill management approach. Attractants and repellents have long been sought for detection, monitoring, and control of the Asian citrus psyllid (ACP), the vector of citrus greening disease. ARS entomologists in Fort Pierce, Florida, discovered that common citrus odors break down (in air) to smaller molecules, two of which highly stimulate ACP antenna. Electroantennography revealed that the common citrus odors ß-ocimene and citral degrade to compounds such as formic and acetic acids, which elicit consistently high antennal responses from ACP. ARS researchers developed a bioassay that allowed ACP adults to probe beads of wax substrate that contained varying proportions and amounts of formic acid, acetic acid, ß-ocimene, and citral. A blend of 2:1 formic:acetic acid elicited the most probing and could form the basis of an attract-and-kill approach for ACP.

New low-herbicide method for Palmer amaranth (pigweed) control. Herbicide-resistant Palmer amaranth threatens cotton and corn production in the United States. The weed is native to the Sonoran Desert and is adapted to intermittent rainfall, making it well-suited for the low water-holding capacity of the coarse-textured soils of the southeastern coastal plain. ARS scientists found that Palmer amaranth changed soil water content to a depth of 1 meter, drawing more than twice as much water per plant as cotton, leading to water stress in nearby cotton plants. Fortunately, ARS scientists also found that high-biomass rolled rye can suppress Palmer amaranth emergence by 50 percent. The time of rye planting in the autumn was the primary factor affecting suppression potential. A timely rye planting in November can provide growers with an alternate control tactic to use in concert with fewer herbicides, thus reducing selection pressures toward the undesirable development of additional herbicide-resistant Palmer amaranth.

Improving methods for evaluating the economics of biocontrol. Biocontrol is a pillar of integrated pest management, but there has been little focus on assigning it an economic value. An ARS scientist in Maricopa, Arizona, working in collaboration with scientists from the University of Arizona, reviewed, synthesized, and analyzed the literature on biocontrol economics. Three forms of biocontrol are currently used: classical (exotic agents are released and allowed to establish), augmentative (control agents are released frequently, as needed, typically using native agents), and conservation (local agents are enhanced via land management practices). The economic value of classical control could be as high as $10,000 gain for each $1 spent, and conservation biocontrol could improve economic benefits by as much as $2,000/hectare, depending on the crop system. This study identified some of the challenges in assigning economic value to biocontrol; it also provided a conceptual outline and a catalog of methods for how economic values can be determined by identifying internal and external costs and benefits. Understanding the economic value of biocontrol will help agriculturalists identify when and where biocontrol is an economically feasible pest control strategy.

New production methods make plant disease biocontrol agent and bioinsecticide widely available. The fungus *Trichoderma* is the most widely used biocontrol agent in the world and effectively controls plant root diseases and improves plant health. *Beauveria bassiana* (spores) is one of the most widely used bioinsecticidal fungi in the world. Both of these are commercially-produced using solid substrate fermentation on moistened grains, which is labor intensive, takes weeks to complete, is plagued with quality control issues, and difficult to scale-up. ARS researchers in Peoria, Illinois, worked with scientists in Brazil and developed low-cost liquid fermentation processes for both of these fungi. For *Trichoderma* the scientists developed a process for producing microsclerotia, which are a very stable fungal form that was unknown in *Trichoderma*. High yields of *Trichoderma* microsclerotia were produced in a 4 day fermentation and were shown to survive drying and remain viable for more than one year when stored at room temperature. Soil incorporation of *Trichoderma* microsclerotia was shown to effectively control damping-off disease in melon seedling. Stable microsclerotial forms of *Trichoderma* will be ideal for use in granular formulations or as seed coatings. For *Beauveria bassiana* the process produced a very high concentration (2-3 x 1012 blastospores/L) of stable, effective spores in only a 3 day fermentation time. Dried spore formulations of this insect-killing fungus showed biological activity when stored at refrigerated temperatures for 1 year or for 4-6 months at room temperature. Use of these production methods will scale-up these products for wide-spread use in agriculture. Commercial development of ARS' patent-pending production processes for these two fungi will provide seed coaters, farmers, organic growers, and homeowners with an effective, nonchemical tool for plant disease control and plant health improvement with *Trichoderma* and an effective, nonchemical insect control tool (a bioinsecticide) with *Beauveria bassiana.*

Improved management of codling moth in apple orchards by trapping with a kairomone lure. Codling moth is the key pest of apple and is a primary pest of pear and walnut. New methods are needed to augment or replace the use of chemical pesticides, particularly in certified organic orchards. ARS researchers in Wapato, Washington, used a 3-chemical kairomone lure in traps to remove females from orchard plots and reduce infestation of apples. At 50 traps per acre in 4-acre plots, the number of apples infested was reduced by 80 percent (compared to control plots). The use of this lure in traps is a feasible method to improve mating disruption and thus reduce codling moth infestations.

Ecological niche model establishing the risk of codling moth spread used to renegotiate agreements and establish new agreements with importing countries. Codling moth is one of the most destructive and economically important pests of apples and a pest of quarantine concern in several regions and countries where it does not occur (e.g., Colombia, Japan, South Korea, and Taiwan). Using new and historical data, ARS scientists in Wapato, Washington, collaborated with scientists at Colorado State University and the Institute of Zoology, Chinese Academy of Sciences, to develop predictive models for codling moth establishment and spread. The models demonstrated that due to the short duration of chilling days and short day length, codling moth could not establish and spread to areas within the 20th parallels. This information is being used by apple industry commodity trade representatives and the Animal and Plant Health Inspection Service (APHIS) to renegotiate agreements and establish new agreements with importing countries where codling moth is of quarantine concern. This will help increase U.S. apple exports to tropical countries without additional postharvest fumigation or cold treatments costs.

StageCast computer model synchronizes pollinators with crop bloom to increase profitability. There is an urgent need for models that help producers synchronize pollinators with crop bloom, especially in crops such as seed alfalfa and almonds. ARS researchers in Fargo, North Dakota, and the University of Idaho–Moscow, developed a modeling environment, StageCast, that provides rigorous statistical support for describing and forecasting the stage specific development of both insects and plants. Following a technical review (ongoing), a beta test version is expected to be made available with a user's guide and related programmer's guide. Using improved Fluctuation Thermal Regime (FTR) protocols developed by ARS and North Dakota State University researchers that will improve synchrony of bees with crops.

Double-stranded ribonucleic acid (dsRNA) molecules delivered to citrus tree foliage control Asian citrus psyllid and a weevil and nontarget effects in foliage and soil are minimal. The use of dsRNA to induce ribonucleic acid interference (also known as gene silencing) is a potentially powerful and highly specific tool for environmentally appropriate control of major pests. ARS entomologists in Fort Pierce, Florida, in collaboration with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, Brazil), demonstrated that dsRNA products caused significant mortality, cessation of feeding, and reduced egg production when Asian citrus psyllid and a weevil fed on treated citrus plant foliage. Tests will be expanded to validate this approach and identify a pathway to a commercial product. The researchers also demonstrated that the dsRNA was not detectable in foliage at three months or soil at 3 days post-treatment. Rapid degradation of dsRNA in the environment and the high degree of specificity of dsRNA for pathogen or insect genes suggest that nontarget effects of exogenously applied dsRNA is not a major concern.

Blocking Asian citrus psyllid (ACP), the vector of citrus greening, salivary sheaths to prevent feeding on citrus and developing improved scent baits with newly identified ACP attractants. Citrus greening disease continues to devastate citrus production in Florida and worldwide. ARS entomologists in Fort Pierce, Florida, identified a strategy to block feeding by the citrus greening vector, the Asian citrus psyllid—inhibiting formation of the insect's salivary sheath. Pure salivary sheaths were isolated and analyzed to identify inhibitors of sheath synthesis that would prevent psyllid feeding when applied to the leaf surface. Sheath disruptors are being evaluated in laboratory and greenhouse trials. These researchers also demonstrated that synthetic compounds that bind with olfactory binding proteins isolated from ACP antennae are attractive to the psyllid and enhanced the attractiveness of naturally-occurring host plant volatiles. These findings will be used to develop scent baits to enhance detection, monitoring, and control of ACP.

Pathogen spore dispenser to suppress Asian citrus psyllid populations. Asian citrus psyllid (ACP) residing in residential areas and abandoned orchards can spread huanglongbing (HLB) to commercial orchards. Insecticidal treatment of these areas is problematic. ARS entomologists in Fort Pierce, Florida, developed a prototype device to dispense spores of pathogenic microorganisms to ACP. Greenhouse tests with free-flying ACP showed that ACP will visit the dispenser and infect other psyllids dwelling on potted citrus trees. The dispenser can be hung from the branches of citrus and other host plants and will be useful for suppressing ACP populations in yards, parks, business zones and abandoned commercial orchards.

Release of flat bark beetles for control of coffee berry borer in Hawaii. Coffee berry borer (CBB) is a serious pest of coffee worldwide and a new invasive pest in Hawaii. Two species of flat bark beetle, *Cathartus quadricollis* and *Leptophloeus* sp., were found inside CBB infested coffee cherries in Hawaii. Using molecular markers, ARS scientists in Hilo, Hawaii, confirmed that these flat bark beetles were predators feeding on CBB. Laboratory assays demonstrated that these predators could feed on all CBB life stages. These predators are widely distributed in the coffee growing areas of the island of Hawaii, but feed mainly in dried coffee on the tree rather than in ripening cherries where crop damage occurs. These species are not susceptible to *Beauveria bassiana* which is used for field control of CBB in coffee. *Cathartus quadricollis* can be raised on a cracked corn-cornmeal diet and a raise-and-release program was started by providing more than 200 coffee farmers on the main island of Hawaii with starter kits.

New monitoring tool for the invasive ambrosia beetle *Euwallacea* nr. *Fornicates*. Avocado and ornamental plants in California, Florida, and Israel are being killed by an exotic ambrosia beetle, *Euwallacea* nr. *fornicates* (Polyphagous Shot Hole Borer), which carries a fungal plant pathogen and is responsible for severe dieback of avocado trees. ARS scientists in Peoria, Illinois, in collaboration with scientists from federal agencies, universities, and commercial entities, identified an attractive compound (quercivorol) from beetle-infested plant material. This compound in combination with survey traps is being used by avocado growers and arborists to monitor the spread of this highly destructive insect pest. The use of the lure (will provide growers with effective detecting tools for implementation in integrated pest management programs.

Component 3 – Protection of Natural Ecosystems

Using semiochemicals to control the invasive Argentine cactus moth in North America. Following the successful eradication of the cactus moth from Mexico using control tactics, including the sterile insect technique, ARS scientists in Tifton, Georgia, and Tallahassee, Florida, continued to develop control technologies for sustainable management of this pest in native desert ecosystems and commercial cactus production areas, including pheromone mating disruption and larval trail-following pheromone disruption. In cooperation with scientists from FuEDEI (Fundación Para el Estudio de Especies Invasivas), Argentina, these ARS scientists conducted mating disruption trials in two plantations in the Argentinian provinces of Catamarca and Santiago del Estero. Pheromone dispensers placed at 1,000/hectare significantly reduced male moths and the number of eggs laid. In cooperation with scientists from SUNY Cortland, New York, laboratory and field trials demonstrated that plant surface treatments with a trail-following pheromone significantly reduced the establishment and survival of cactus moth larvae. Results from these trials, using semiochemicals as a control tactic, are encouraging further development of two management tools for commercial cactus plantations.

Discovery and use of parasitoids for biological control of the invasive emerald ash borer (EAB), *Agrilus planipennis Fairmaire*. Biological control of an invasive pest such as the emerald ash borer (EAB), *Agrilus planipennis Fairmaire*, is predicated on the discovery and evaluation of co-evolved natural enemies from the pest's native range. ARS scientists in Newark, Delaware, identified several parasitoids for biocontrol of the beetle. The researchers discovered *Spathius galinae,* a larval parasitoid that originated from the Russian Far East (EAB's native range), in 2010, described it as a new species in 2012, and reared and tested it for its host specificity. Based on this testing and other biological evaluations, this parasitoid was approved for release as an EAB biocontrol agent. The parasitoid *Tetrastichus planipennisi* Yang and two other Asian-origin EAB parasitoids were released for biological control between 2007 and 2010. These ARS researchers, Forest Service scientists, and University of Massachusetts researchers observed around a 90 percent decline in densities of EAB larvae in infested ash trees in aftermath forests in southern Michigan, where they were released. These results demonstrate that effective EAB biological control strategies in North America should focus on establishing stable populations of introduced specialist parasitoids for sustained suppression of EAB populations. This biological control strategy has been adapted in 19 EAB-infested states. This research has provided private land owners, federal, state, and local park or land managers, foresters, and researchers with new EAB biocontrol agents and a strategy for identifying additional ones.

Adaptive management tools for areawide waterhyacinth control. Waterhyacinth is considered among the worst floating aquatic weeds on earth. It has become especially problematic in Florida and the Sacramento–San Joaquin River Delta in California by impeding navigation and mosquito abatement and hindering the use of scarce water resources in a time of severe drought. ARS scientists and collaborators have developed two new tools to improve control of waterhyacinth on an areawide basis. First, an accurate mapping application was developed that uses Landsat multispectral satellite images and automated image processing to detect water hyacinth among other species of floating plants. The application tracks seasonal development of waterhyacinth populations allowing pest control operators to track their success and to target nursery populations. Second, a new biological control agent against waterhyacinth, the planthopper insect *Megamelus scutellaris*, has been identified and is now being produced and released. Nearly 400,000 of the insects have been released throughout Florida and a robust population has been established in the Sacramento River watershed. Damage inflicted by biological control agents make the plant more susceptible to other control methods. These new tools help make possible successful, adaptive, and integrated management of water hyacinth and other aquatic weeds.

Hybridization of herbicide-resistant pigweed exacerbates weed control problems. Weedy amaranth plants (pigweed), especially herbicide-resistant biotypes, are problematic in many crops. ARS scientists have shown that hybridization between Palmer and spiny amaranth occurs, and these naturally occurring crosses can transfer acetolactate synthase (ALS)-inhibitor genes, weed genes that confer glyphosate resistance. Glyphosate-resistant Palmer amaranth populations in the Mississippi Delta contain two different ALS point mutations, each conferring resistance, but only one was found in the hybrids. The ALS point mutations have been sequenced and are the type that gives broad spectrum resistance to all five classes of ALS inhibitor herbicides and resistance to four different herbicides was confirmed in greenhouse tests. Therefore, herbicide resistance genes can move among amaranth species and further use of glyphosate and other ALS herbicides will result in a proliferation and spread of glyphosate resistance to multiple amaranth weeds.

Biological control of giant reed. Giant reed (also called arundo and carrizo cane) is an aggressive invader from Europe that forms dense stands twenty feet high along streams. This reed creates a security risk for the U.S. Customs and Border Patrol along the Rio Grande Valley of Texas, increases disease-spreading ticks, increases the risk of flooding and wildfire, and uses precious water resources in the semiarid western United States. ARS scientists have developed a biological control program using the arundo wasp. This insect has reduced the above ground biomass of cane by 20 to 50 percent between Del Rio and Brownsville, Texas. This equates to 2.5 million tons of cane removed from the river and 6,000 acre-feet of water conserved each year (worth $4.4 million). The cane is slowly declining and ARS researchers have developed a cane topping technique which accelerates this decline. This management strategy is being implemented with partnerships that include the Department of Homeland Security, Texas state agencies, and Mexico.

Lower costs and increased predictability of success lead to enhanced adoption of ecologically-based restoration of sagebrush steppe. Over 14 million acres of sagebrush steppe in the Western United States have been identified as high priority for restoration. These lands provide critical habitat for sage grouse, a species considered for listing under the endangered species act. However, rangeland restoration efforts have had a high failure rate, and combined with the expense of seeding, this has dramatically hindered its adoption. ARS scientists in Burns, Oregon, and partners have identified key ecological processes that include seed and seedling pathogen attack, winter drought, seed predation, herbivory, freeze/thaw cycles, and soil physical crusts that determine seeding success and/or failure in rangeland restoration. As a result of this research, new management strategies have been developed to better predict the success (lower the uncertainty) of restoration projects' outcomes and reduce their costs.

Component 4 – Protection of Postharvest Commodities and Quarantine

Methyl bromide alternatives developed for strawberry production. Strawberry growers have had to shift away from using methyl bromide for preplant soil treatments and postharvest quarantine. Fusarium wilt is a soil-borne plant disease that has increased to new levels of significance with the elimination of methyl bromide soil treatments. ARS scientists identified strawberry cultivars with high levels of genetic resistance to Fusarium wilt. The strawberry plant varieties Fronteras, Portola, and San Andreas were among the most resistant, whereas others such as Albion and Monterey were much more susceptible. After berry harvest, ARS scientists improved fruit quality during storage and quarantine using short treatments of nitric oxide. Nitric oxide use helped control quarantined pests such as western flower thrips and improve postharvest storage life. These new strategies for pest control provide the means for continued production and export of high-quality strawberries without the need for methyl bromide.

Improvement of naval orangeworm control methods for almonds and pistachios. Pyrethroid insecticides have been the primary tool used to control navel orangeworm in almonds and pistachios, but resistance was recently reported in Kern County, California. A team of ARS and university researchers found that the mode of action for resistance was an enhanced activity of the cytochrome P450 oxidase system, which conferred a 10- to 15-fold increase in resistance. ARS scientists also determined that both diamide and spinosyn insecticides will kill or paralyze adult orangeworm, with the maximum effect at 48 hours, thus providing new tools for insecticide rotation strategies to delay further resistance development. In addition, phenyl propionate was identified as a nonpheromonal attractant that could be combined with a known pheromone attractant to improve trap capture, thereby improving monitoring systems—a key element for effective integrated pest management. Improved control will enhance the quality and yield of these tree nuts.

Creation of male-only strains improve the sterile insect technique (SIT) in the Mexican fruit fly for better control. A transgenic tetracycline-suppressible female-specific embryonic lethality strain in the Mexican fruit fly (Mexfly) was created for male-only strains to improve the SIT. Parental Mexfly females fed a tetracycline-free diet gave rise to progeny that were more than 99 percent male by the first larval instar. The results suggest that a gene promoter that drives embryonic lethality in this system may also function maternally in developing oocytes of the Mexican fruit fly. This has resulted in a Mexican fruit fly strain that exhibits both female sterility and/or female-specific lethality, providing a better strategy for controlling this important agricultural pest insect.

Biological control of white peach scale in Hawaiian papaya. White peach scale is a serious economic and quarantine pest of papaya in Hawaii. Scale insects are good targets for classical biological control. ARS scientists in Hilo, Hawaii, imported the highly specialized parasitic wasp *Encarsia diaspidicola* from Western Samoa and released it in 2013 after extensive host testing. Trapping studies demonstrated that the wasp has established itself in Hawaii and is spreading naturally through the main papaya production area on the island of Hawaii. Farmers are helping to spread the biocontrol agent by moving papaya logs infested with parasitized scales to new fields.

Adjusting the particle size of aerosol applications can maximize insect control. The use of aerosol applications of reduced risk insecticides, as an alternative to fumigating food facilities with methyl bromide to control insects inside flour mills, has been rising. Efficacy of aerosols can be strongly impacted by the size of the droplets produced, but little information is available on the relationship between droplet size and efficacy against stored product insects, and the droplet sizes produced by different application systems. ARS scientists in Manhattan, Kansas, demonstrated that pyrethrin aerosol applied as 16 micron droplets was far more effective against adult confused flour beetles then 2 micron droplets. Industry personnel applying aerosols can adjust aerosol dispensing equipment so that larger particles are dispensed. This could maximize control of flour beetles inside mills and food warehouses.

Sequencing the lesser grain borer genome to help develop new biopesticides for its control. The lesser grain borer is an economically important storage pest worldwide. In a number of locations, there have been reports of resistance in the grain borer to the primary fumigant used to control it, phosphine. Genetic targets would facilitate development of new biopesticides to replace phosphine. Strains of the lesser grain borer were inbred for more than 30 generations by ARS researchers and genomic data was acquired from one inbred strain through various sequencing platforms, including PGM, Illumina, and PacBio. ARS researchers are polishing the genome sequence and will annotate the genome through the i5k insect genome project. Once the genome is completed, specific genes can be targeted to disrupt metabolic pathways in the lesser grain borer. Researchers and cooperators in private industry can use these results to speed up the development process of new biopesticides with specific activity against the lesser grain borer.

Using meta-analysis to evaluate structural treatments' pest control efficacy in food processing facilities. Due to the phase out of methyl bromide (for the treatment of flour mills) the effectiveness of alternative structural treatments for managing important pests needs to be evaluated. An analysis of the treatments was completed using meta-analysis techniques, a statistical procedure that combines the results from multiple independent studies. Structural treatments (fumigation with sulfuryl fluoride or methyl bromide, or heat) greatly reduced pest populations in food facilities; all treatments provided similar initial reductions in beetle abundance. This was the first large-scale analysis of pest population trends and management tactic efficacy in commercial food facilities. Results showed that structural treatments are initially effective but differences between types of facilities must be accounted for when developing control programs. Also, this novel application of meta-analysis provided insights into potential efficiency impact factors and identified data gaps.

Genetic resistance to *Fusarium oxysporum* f. sp. *fragariae* among strawberry cultivars identified. As strawberry growers have had to shift away from the use of methyl bromide for preplant soil treatments, certain soilborne disease problems, including Fusarium wilt of strawberry, have become more significant. To develop proper disease management strategies it is important to understand if pathogen resistance varies among strawberry cultivars and germplasm. In field trials, University of California-Davis and ARS scientists determined that some strawberry cultivars are highly resistant to the *Fusarium oxysporum* f. sp. *fragariae* (Fof) pathogen, including San Andreas, Portola, and Fronteras, whereas others, including Albion and Monterey, are much more susceptible. The information will help strawberry growers avoid Fof-susceptible cultivars, and it will help strawberry breeders develop new lines of resistant strawberry plants.