National Program 304:
Crop Protection and Quarantine

ACCOMPLISHMENT REPORT 2007-2012

United States Department of Agriculture

Research, Education, and Economics

AGRICULTURAL RESEARCH SERVICE

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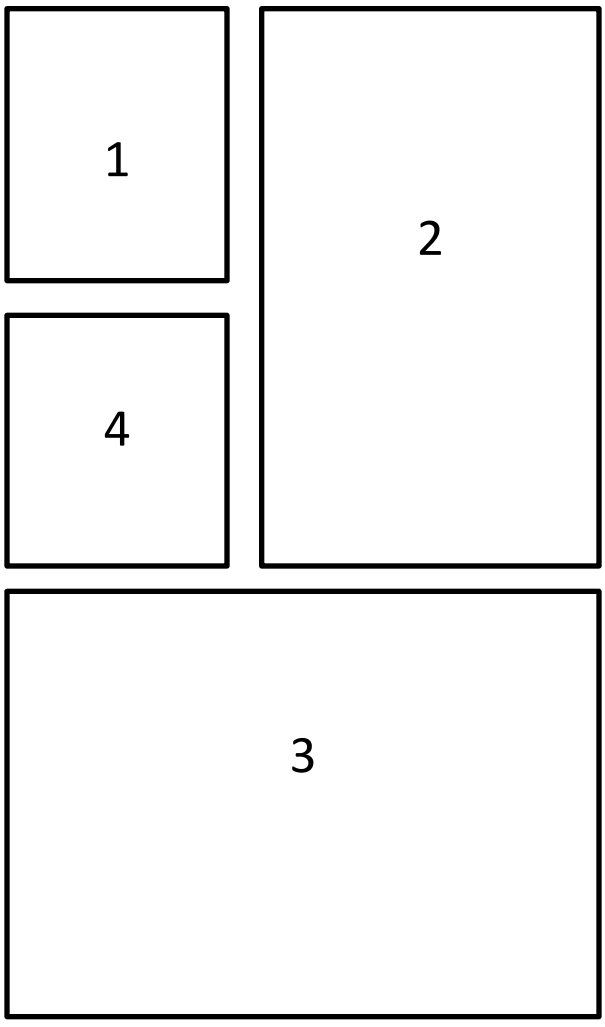
Captions of front page photos, clockwise from upper left:



1. The Asian longhorned beetle, *Anoplophora glabripennis*, is an invasive insect pest of hardwood trees (particularly maples) and a $670 billion threat to the United States. The research of ARS scientists is improving survey and detection of the beetle, the most costly and important component of successful management. *Photo by Matt Smith/ARS.*
2. The paperbark tree, *Melaleuca quinquenervia*, is an invasive species that significantly decreases natural diversity. ARS scientists in the United States and overseas discovered three biological control agents that eat the paperbark tree and released them as part of a successful integrated management plan that led to a nine-fold increase in native plant species richness. *Photo by Albert (Bud) Mayfield, USDA Forest Service, Bugwood.org*, *Center for Invasive Species and Ecosystem Health*.
3. This adult female (top right) and immature 5th instar nymph (lower right) of the beneficial predatory spined soldier bug, *Podisus maculiventris* (Say), are displaying biological control of insect pests as they feed upon a 4th instar of the cabbage looper, *Trichoplusia* ni (Hübner). *Photo by Tom Coudron/ARS.*
4. The invasive spotted wing drosophila (*Drosophila suzukii*) threatens the ability of growers in the Western United States to export California grown table-grapes and sweet cherries to Australia. ARS scientists developed a combination of sulfur dioxide fumigation and cold-treatment as a postharvest alternative to methyl bromide fumigation for controlling this pest, retaining and expanding market access to Australia estimated at $55 million annually. *Photo by Hannah Burrack, North Carolina State University, Bugwood.org*, *Center for Invasive Species and Ecosystem Health*.

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BACKGROUND AND GENERAL INFORMATION

The USDA Agricultural Research Service (ARS) National Program 304, Crop Protection and Quarantine, is the Nation's cornerstone for protecting agriculture and natural ecosystems from insect, mite, and weed pests. Management of these pests is critical for maintaining a safe, high-quality supply of food and fiber and for providing healthy urban landscapes, forests, wetlands, and aquatic ecosystems. The vision of National Program 304 (NP 304) is to develop sustainable pest management systems, with pests defined as both insects and weeds.

Justification for this program is based on major challenges that U.S. crop production and natural ecosystems face from both native and invasive pests. Food and fiber crops worth more than $237 billion and natural ecosystems with incalculable value must be protected from these pests. Challenges are dynamic due to the adaptations of traditional pests to control measures, frequent emergence of new pests, unknown effects of climate extremes on pest development, and public pressure to reduce the use of chemical pesticides. Also, increased global trade and travel have led to increasing numbers of invasive species, causing major environmental damage and losses totaling approximately $137 billion per year. Pest management and control systems must adapt to these challenges to maintain the productivity and competitiveness of U.S. agriculture and the viability of natural ecosystems.

The 204 scientists on the 93 projects associated with NP 304 conduct fundamental and applied research with the aim of improving strategies for the cost-effective management and control of pests while minimizing impacts on the environment and human health. They work in 46 laboratories located in 23 States and the District of Columbia in the United States and at the five ARS Overseas Biological Control Laboratories located in Montpellier, France; Thessaloniki, Greece; Hurlingham, Argentina; Brisbane, Australia; and Beijing, China. The overseas laboratories serve as bases for discovery and development of biological control agents and work closely with ARS scientists in the United States on approximately 50 projects to categorize, evaluate, and test insects and pathogens that might restore

balance between an invasive species and natural controls. Those projects are supported by seven ARS quarantine facilities that allow scientists to test the safety and potential efficacy of biological control agents before they have been approved for release. This ongoing collaboration and coordination among ARS scientists helps to maintain a robust program for the development of biological control agents as sustainable, cost-effective, and environmentally compatible solutions to important invasive weeds and pests.

The principal strategy of NP 304 is to employ integrated pest management (IPM) approaches that utilize cultural, biological, physical, and chemical methods. Although, some pests are still best managed with synthetic chemicals, there is a need to develop and use more sustainable control methods, with emphasis on biological and cultural controls and plant-incorporated protectants.

Many of the insect pests studied by NP 304 scientists are well known to the general public and often newsworthy. For example, the brown marmorated stink bug—in addition to being a serious pest of vegetable crops, fruit trees, and ornamentals—is a nuisance pest that commonly aggregates in homes. The invasive tree pests—emerald ash borer and Asian longhorned beetle—threaten to decimate the biodiversity and the vitality of urban landscapes and forest systems. The Asian citrus psyllid transmits citrus greening disease (Huanglongbing), which threatens to eliminate the citrus industry in the United States.

Noxious weeds are no less problematic. A small minority of weeds cause disproportionately large problems in agriculture and ecosystems, with estimated loses of at least $100 billion dollars per year in the United States. Farmers are constantly challenged with weeds, especially those resistant to herbicides. Terrestrial weeds such as Old World climbing fern, saltcedar, and paperbark tree wreak havoc on ecosystems, and aquatic weeds such as hydrilla and water hyacinth degrade fish and waterfowl habitats and restrict the movement of recreational and commercial vessels.

Overall, the weed and insect pests investigated by NP 304 scientists are vitally important because they threaten more than 250 million acres of food and fiber crops and vast areas of natural ecosystems. Agricultural pests affect farm-gate values, rural economies, and agricultural exports, while pests of natural ecosystems impact biodiversity, outdoor recreation, and dependent industries. Ultimately, all of these pests affect the Nation's economy.

Between 2007 and 2012, NP 304 scientists significantly advanced systems and technologies for controlling pests, as documented in this report. Reflecting the need to meet a broad diversity of challenges, technologies and products generated by NP 304 scientists during the last 5 years are highly varied and include biological agents to control invasive weeds and insect pests, pheromones and other attractants to monitor insect pests and for insect mating disruption systems, reference aids for identifying potential insect pests at ports, biocompatible foams for delivering microbial agents for insect control, and the genome sequencing of the important post-harvest pest beetle, *Tribolium castaneum*. ARS scientists made several breakthrough discoveries for controlling insect pests, such as a newly commercialized entomopathic bacterium, *Chromobacterium subtsugae,* that kills many key piercing-and-sucking plant pests not controlled by other biopesticides, such as *Bacillus thuringiensis* (Bt). In addition, they have developed a new insecticidal mode-of-action based on insect neuropeptides. These discoveries lay the foundation for the next generation of more environmentally

sensitive insecticides. Furthermore, ARS biocontrol technology has successfully transformed wetland landscapes, especially in the Florida Everglades, which was once dominated by the aggressive weed, *Melaleuca* paperbark tree.

The researchers in this National Program are also leading the development of new crop protection and quarantine tools. For example, in arthropod genomics, ARS, with multiple university collaborators, is leading an effort (the i5K Initiative) to sequence the genomes of 5,000 insects, mites, and other arthropods of critical importance to agriculture (http://arthropodgenomes.org/wiki/i5k). For NP 304, this sequence information will enable researchers to develop new pest-specific research and management tools. One of these tools is RNA interference (RNAi) technology (gene silencing), an exceptionally promising area of research that is dependent on knowledge of the insect genome. ARS scientists were among the first to realize that RNAi could be used to kill insects by shutting down essential protein production. NP 304 scientists then showed that orange trees and potatoes take up RNAi systematically, killing the target insect (in this case the psyllids that transmit the pathogens of citrus greening and zebra chip) that feeds on the plant. The RNAi process has been used by ARS and others as a plant-incorporated protectant in transgenic crop species. Of particular significance, ARS researchers in NP 304 conducted the first successful field demonstration of RNAi technologies to control a virus that kills the honey bee and that were subsequently commercialized by an industry partner. ARS scientists are working with industry under many cooperative research and development agreements to develop more such products. Use of this type of flexible public-private partnership approach enables ARS to more effectively address urgent problems.

PLANNING AND COORDINATION FOR THE NP 304 5-YEAR CYCLE

Prior to the start of the current 5-year research cycle, ARS scientists and administrators held a workshop in May 2008 with customers, stakeholders, and research partners to identify major crop protection and quarantine agricultural issues and research priorities. At the workshop, four major research themes were formulated as the basis for programmatic research planning: systematics, crop protection, natural ecosystem protection, and post-harvest protection and quarantine. These themes were used as the basis for the four components in the NP 304 Action Plan.

Following the 2008 workshop, a writing team composed of ARS scientists and National Program Leaders (NPLs) drafted the current NP 304 Action Plan. Incorporating input from customer/stakeholder interactions, knowledge of scientific subject matter, and input from other ARS scientists and cooperators, the writing team identified the top priority needs that ARS could address with its research. These individual research needs were aggregated into problem statements under each of the four research components. The Action Plan guided development of new individual NP 304 research projects that began the new 5-year research cycle in 2008.

All of the individual research plans for NP 304 projects include statements of the agricultural problem being addressed, the anticipated products or information to be generated by the project, how the planned research contributed to mitigating or solving the larger National Program problem statements, and timelines and milestones for measuring progress toward achieving project objectives. All of the

NP 304 project plans were evaluated prospectively for scientific quality by external peer review panels. Project plans then were revised in response to review panel recommendations, if needed, and implemented.

The next 5-year cycle for NP 304 will begin with a new Action Plan to be written in January–February 2014, and new project plans will be developed in mid-2014.

Coordination and planning for NP 304 are the tasks of the NPLs who constitute the NP 304 leadership team. The NPLs also coordinate NP 304 activities with other ARS National Programs and with other agencies and departments. Within the USDA, ARS researchers collaborate with the Animal and Plant Health Inspection Service (APHIS), the National Institute for Food and Agriculture, the U.S. Forest Service, the Foreign Agricultural Service, and the Natural Resources and Conservation Service. Important inter-departmental collaborations occur with the Department of Homeland Security (DHS); the Department of Interior, the Department of Defense; the Department of State, particularly the U.S. Agency for International Development; and with the U.S. Environmental Protection Agency. All Agency work is coordinated with the Office of Science and Technology Policy, and through the Office of Management and Budget.

Customer and stakeholder interaction and research coordination have continued to play important roles in helping NPLs guide NP 304 research to maintain its relevance to U.S. agriculture. As shown in Appendix 3, NP 304 NPLs attended or organized numerous research planning, coordination, and stakeholder workshops during 2007–2012. These workshops addressed crop/commodity-specific challenges, emerging high-priority issues, and new scientific developments in the fields of entomology, weed science, genetics, microbiology, systematics, and insect and plant genomics.

STRUCTURE OF NP 304

The NP 304 Action Plan includes the four Research Components listed below. The full Action Plan can be viewed online at: www.ars.usda.gov/research/programs/programs.htm?np_code=304&docid=17844.

Component 1: Systematics and Identification.
For this component, ARS efforts focus on the identification of insects and weeds that are potential pests of the Nation's crops and natural ecosystems. Insects and microbes that are possible natural enemies of invasive pests also are identified. Systematics tools, including cladistics and DNA bar-coding, are used to categorize insects based on genetic and evolutionary relationships. Taxonomic revisions are conducted, including description of new species. Confirmatory identifications are made for potentially invasive insects and weeds.

Component 2: Protection of Agricultural and Horticultural Crops.
In this component, ARS scientists develop innovative approaches to control arthropod pests and weeds of field and horticultural crops. Pests of multiple crops are investigated, ranging from established native pests to recently introduced invasive pests. Biological and cultural control methods are emphasized, but efforts include developing more environmentally friendly chemical methods. Resistance management programs are employed for genetically engineered crops with plant-incorporated protectants. Scientists explore novel ways to control pests

utilizing technological advances in molecular genetics, proteomics, physiology, biochemistry, and genomics. Scientists often utilize expertise and resources from ARS Overseas Biological Control Laboratories.

Component 3: Protection of Natural Ecosystems.
This component encompasses ARS' efforts to control insect and weed pests of urban landscapes, forests, wetlands, and aquatic ecosystems. Scientists focus on the most economically important pests. Many ecosystems where these pests occur consist of huge areas with high environmental value, so management options are limited to biologically based approaches, including use of semiochemicals in trapping systems, sterile insect techniques, and biological control. Scientists often collaborate with the ARS Overseas Biological Control Laboratories to find and characterize potential biocontrol agents.

Component 4: Protection of Post-Harvest Commodities and Quarantine.
Research in this component strives to improve methods for reducing pest damage in fresh, stored and processed commodities and limit the spread of exotic pests. Scientists develop sensitive acoustic or electromagnetic tools to cost-effectively and accurately detect pest infestations and systems to suppress or eradicate invasive insects, especially fruit flies and moths. Depending on the pest problem, scientists use sterile insects, biological control, attractants and traps, or a combination of these techniques to control or eradicate pests.

ARS is a mission-directed, problem-solving agency with the capacity to integrate basic and applied research in response to stakeholder needs. Timelines for developing technologies and products vary greatly. Many research needs are often urgent and specific, such as the need to develop an attractant for the brown marmorated stink bug, whereas other requests are long-term and complex, such as the need to eradicate cactus moth from Florida. As in these examples, most of the research conducted by NP 304 scientists is a blend of basic and applied research directed toward controlling pests. Figure 1 illustrates the fundamental stages toward developing effective pest control: Detection, Assessment, Solutions, Implementation, and Evaluation. NP 304 scientists are closely involved with each of these stages, depending on the pest problem. In the case of a new pest, such

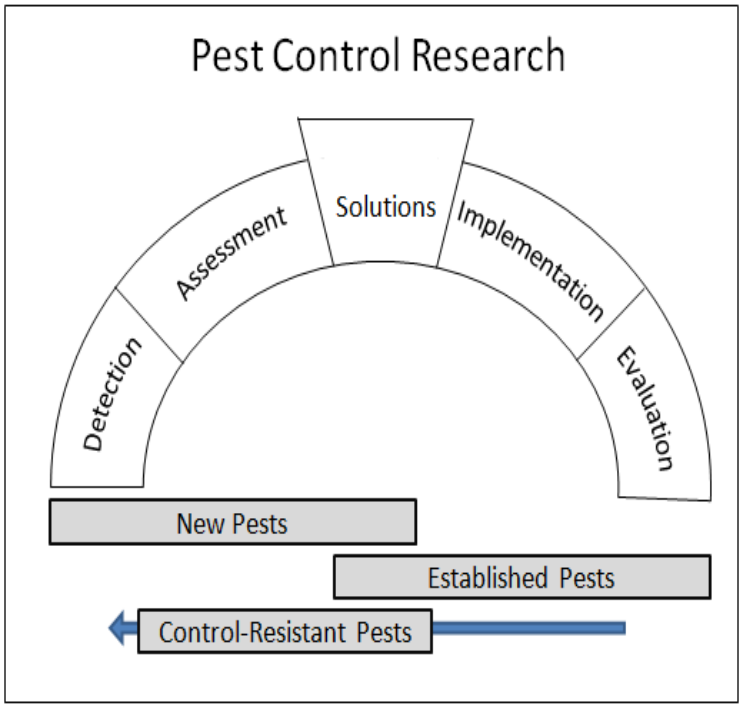

Figure 1: Pest control research has five interconnected stages, illustrated as blocks of an arch. ARS scientists contribute to all stages, but specialize in developing solutions, the keystone.

as spotted wing drosophila, the emphasis is on detection (defining range of occurrence), assessment (characterizing pest impact), and solutions (problem solving), whereas for an established pest such as the paperbark tree (*Melaleuca*), emphasis is on biocontrol solutions, implementation with technology transfer, and post-treatment evaluation.

The status of a pest can change; for example, in some cases, herbicide-resistant weeds, have moved from "established" pests when they were controlled by traditional methods to "new" pests that require additional attention by scientists (Figure 1, arrow). ARS regularly communicates with scientists from other agencies, especially APHIS, to determine the status of potential invasive pests and, when appropriate, begin research to address problems. In some cases, ARS conducts research in support of APHIS eradication (e.g., Asian longhorned beetle), while, in others, it develops long-term management strategies for farmers and land-managers.

The goal of ARS research is to develop and transfer cost-effective pest-control Solutions (systems, technologies, and methods) for implementation. For example, the DHS U.S. Customs and Border Protection approached ARS in 2007 about a problem in the Rio Grande Valley with giant cane (*Arundo donax*). The cane was growing to 20 feet in height in dense masses that prevented Border Patrol officers from having adequate visibility. These stands of giant cane can cover many square miles of area along the river, not only creating a security problem, but also removing water from the ecosystem and sheltering the invasive cattle fever tick. ARS scientists in Weslaco, Texas, worked with researchers at the ARS European Biological Control Laboratory to develop biocontrol solutions to the problem. Together, they identified insect biological control agents from the giant cane's native range in southern Europe, which have now been released and are creating areas where visibility through the canes has increased to an acceptable level. As a second example, ARS technologies were transferred to organic farmers in Florida, who received training in the use of sunflowers as a trap crop in a push-pull strategy to control thrips on peppers and tomatoes.

ARS systematists addressing Component 1 primarily contribute to the Detection stage because they are on the front line of pest identification, but they also contribute to identifying possible biological control agents in the Solutions stage. Through the Smithsonian Institution and other museums, these scientists maintain premier insect, mite, and weed reference collections and have developed expertise in identifying new pestiferous arthropods and weeds. Scientists addressing research in Components 2, 3, and 4 assess the impact of pests (Assessment stage) and ultimately implement control measures (Implementation stage), but their specialty is developing solutions for controlling pests defined within their components. These solutions rely on a solid foundation of basic research and require integration of multiple disciplines, technologies, and tools to develop cost-effective and environmentally friendly pest control systems (Figure 2).

NP 304 research is organized by pest complex, commodity, or ecosystem, depending on the situation, but also by cross-cutting issues. For example, corn growers and cotton growers have important insect pests and weed problems that are addressed within crop production systems; but cross-cutting issues such as managing insect resistance to crops—especially genetically engineered crops—or weed resistance to herbicides, benefit from sharing pest resistance management strategies across several crops. This helps improve research approaches and reduce redundant research and allows scientists to quickly share innovative solutions or strategies.

Other cross-cutting issues used to coordinate NP 304 projects include invasive species, environmental risk management, climate change, and biofuels, among others. Lastly, the emergence of insect and weed genomics provides a cross-cutting platform for organizing pest research as scientists use genomes to explore pest vulnerabilities and new control methods.

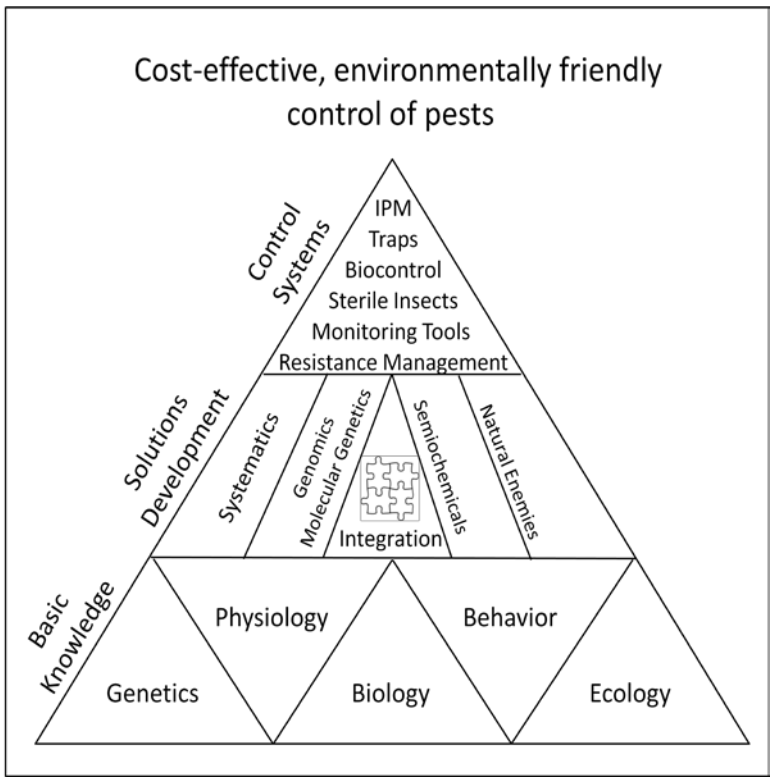

Figure 2: Like a pyramid, research products require a broad foundation.

Budget reductions over the past few years have prompted the realignment of some research priorities within NP 304 and the closure of several laboratories that supported NP 304 research. The laboratories in Fairbanks, Alaska; Shafter, California; and Weslaco, Texas, were among the most prominent. Fortunately, many of the experienced researchers and support staff at those locations were able to relocate to other laboratories to continue to provide their expertise to ARS research. In addition, some projects that were originally in NP 304 were combined with related projects in other National Programs. The research contributions from those closed laboratories and combined projects are reflected in this report, and are noted in the publication list in Appendix 2.

For organizational and administrative purposes, specific NP 304 research projects are assigned to only one of the four research components. However, many research projects contribute to the goals of multiple NP 304 research components or to other National Programs.

RELATIONSHIP OF NP 304 TO OTHER NATIONAL PROGRAMS

ARS research is organized into four National Program areas: Nutrition, Food Safety, and Quality; Animal Production and Protection; Natural Resources and Sustainable Agricultural Systems; and Crop Production and Protection (CPP).

The CPP program area is home to four National Programs:

- NP 301 – Plant Genetic Resources, Genomics, and Genetic Improvement
- NP 303 – Plant Diseases
- NP 304 – Plant Protection and Quarantine
- NP 305 – Crop Production

Projects from a fifth CPP National Program—NP 308, Methyl Bromide Alternatives—were merged into NP 304 and NP 303 in FY 2012. The NP 308 projects merged into NP 304 support research in Component IV. The accomplishments from NP 308 projects for the 2006–2011 time period were included in a report prepared in September 2011 at: (www.ars.usda.gov/SP2UserFiles/Program/308/NP308%20ACCOMPLISHMENT%20REPORT%20FINAL%2009%2015%202011.pdf).

Collaborations among scientists within CPP National Programs are common because all of the programs, directly or indirectly, benefit from controlling agricultural pests. For example, collaborations with plant breeders (NP 301) produce plants naturally resistant to insect pests, technologies developed with plant disease scientists (NP 303) reduce plant diseases vectored by insects, and insect control technologies developed with crop production scientists (NP 305) lead to healthier crops with higher yield and quality. NP 304 also collaborates with National Programs outside of the CPP area, including National Program 212–Climate Change, Soils, and Emissions, which investigates the effects of climate change on weeds (Figure 3).

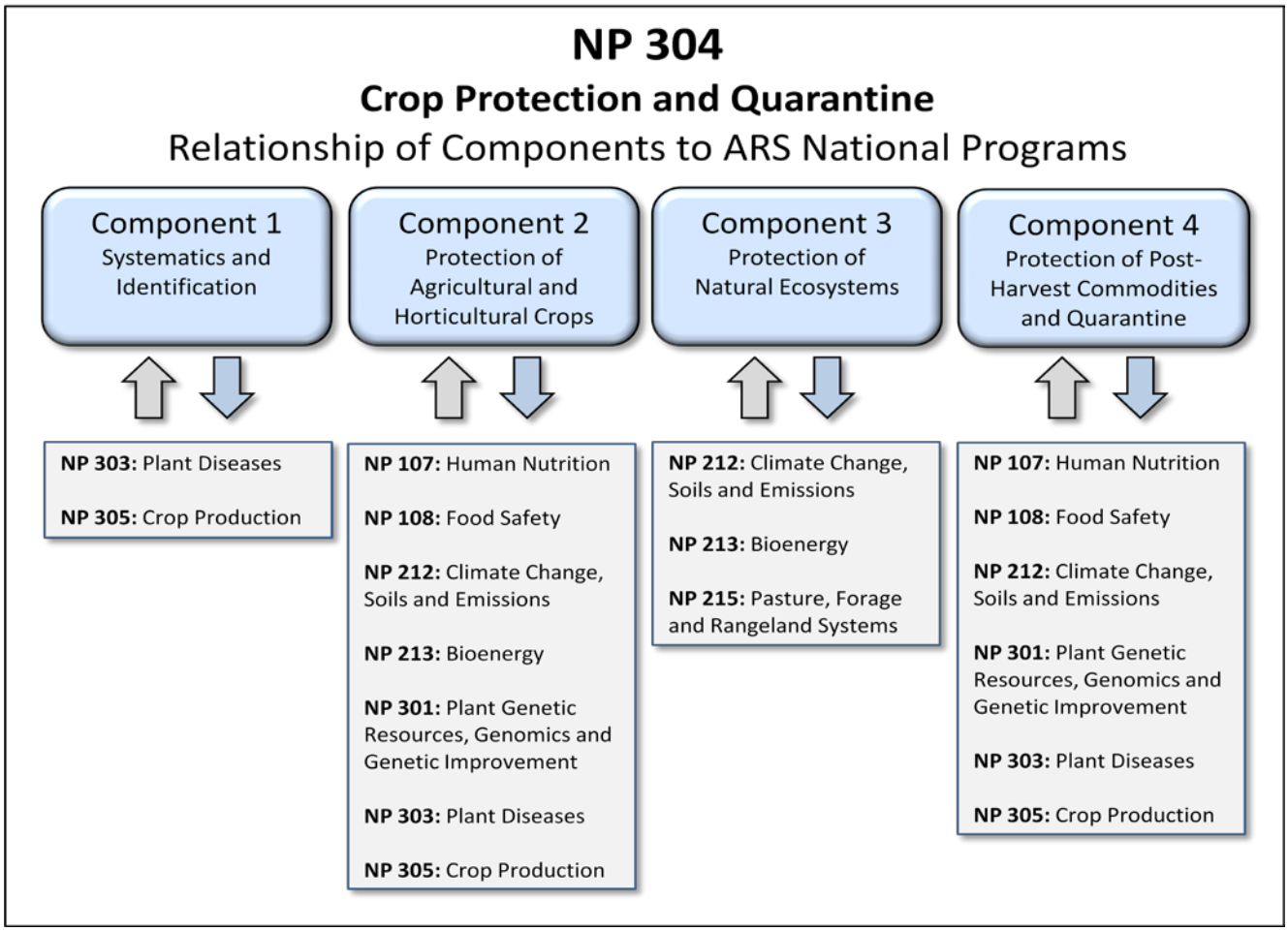

Figure 3: Associations among other ARS National Programs both contributing to and receiving assistance from the four components of National Program 304, Crop Production and Quarantine.

In addition, NP 304 accomplishments are often achieved in close cooperation with public and private sector collaborators. In fact, many NP 304 researchers are co-located on land-grant university campuses, an association that strengthens the research capacities of both institutions. These partnerships enable ARS researchers to participate in and contribute to the training of America's future agricultural researchers and entrepreneurs. The training and preparing of young scientists for careers in agriculture is critical for sustaining modern agriculture. The following table shows that during this 5-year period NP 304 scientists trained and mentored nearly 750 postdoctoral fellows and graduate and undergraduate students. This is an important measure of the leadership and community stewardship NP 304 research leaders and scientists are contributing to agriculture and related sciences.

Visiting Scientists and Students with National Program 304 Projects

Visiting Scientists (over 3 months)	Postdoctoral Fellows	Graduate Students	Undergraduate Students
48	86	182	479

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

The NP 304 Accomplishment Report is a distillation of some of the most significant accomplishments of the past 5 years by ARS scientists working on the National Program's goals. It is a snapshot of the research conducted during this period and its early benefits. In a report on the value of agricultural research (www.ers.usda.gov/publications/eb10/eb10.pdf), the USDA Economic Research Service pointed out that the benefits of research usually trail its completion by 5 to 10 years because it often takes that long before the technology is completely developed, transferred, and adopted by end-users. Thus, the accomplishments and breakthroughs in this report are unlikely to reflect all of the resulting impact of the research.

In this report, NP 304 accomplishments and their impact are organized and presented according to research components and their linked problem statements as described in the Action Plan. The content of this accomplishment report was derived primarily from NP 304 scientists' summaries of their projects' highest impact accomplishments from the 2007–2012 NP 304 annual reports and other reports of NP 304 research projects. This report focuses on the actual or projected impacts of those accomplishments and, where relevant, cites key publications or Web URLs documenting those accomplishments. NP 304's current research projects are listed in Appendix 1. Publications authored by NP 304 scientists are compiled in Appendix 2; publications that document specific accomplishments are also cited within the narrative text. Appendix 3 lists patents issued during this report period, information about how NP 304 research aligns with the ARS Strategic Plan, and research and planning coordination meetings. Appendix 4 contains a reference table matching accomplishments with their related Action Plan Problem Statements.

This report was prepared for an external (to USDA) retrospective review of NP 304 to assess how well this National Program attained its projected goals, as outlined in its current Action Plan. Accordingly, the purpose of the retrospective review is not to judge the performance of individual NP 304 research

projects, but to gauge the overall impact of the National Program. Consequently, the report does not attempt to catalogue all the individual accomplishments of NP 304's constituent research projects. Individual scientists or projects are not identified by name in the narrative text; rather, their achievements are described in the context of contributions towards accomplishing the National Program's stated commitments to effective insect and weed management.

COMPONENT I: Systematics and Identification

The identification of arthropods, plants that are out of place, and the microbes that interact with all aspects of agriculture is an obvious need for research and for the practice of agriculture. Knowing how to control an insect and what damage to expect from a given weed or utilizing a microbe as a biocontrol agent depends on accurate identification of that organism. The involvement of the USDA in identifying and classifying organisms goes back to the Department's origins, when it employed the largest corps of entomologists of the 19th century to begin the enormous task of classifying insects in the United States. Those efforts have continued and have expanded to include weeds and microbes.

The discipline of identification, or taxonomy, is a subset of systematics. Systematics is the science that studies the diversity of organisms, including not only identification, but also genetic and evolutionary relationships between different kinds of organisms. For ARS, the simplest relationship between systematics and taxonomy is that systematics provides the biological basis for meaningful classification and naming. Therefore, even basic developments in systematics can eventually result in practical improvements in identification. An immediate example of this relationship is the use of barcoding to identify species by matching short sequences of mitochondrial DNA with sequences typical of that particular species. This convenient method requires the study of genetic relationships among species in a particular group in order to understand the variation and consistency of the sequence within the species as well as its uniqueness among species.

Over the past 5 years, ARS scientists have performed a wide variety of research projects in this field, ranging from confirmatory identification of potentially invasive insects at the Nation's ports and airports to theoretical studies that have changed the framework of the science of ecology. Studies with insects, in particular, have supported revisionary taxonomic work, including descriptions of new species and their rearrangement into natural classifications. Research on weeds and microorganisms is more limited and focused on support of other work, such as control of invasive weeds and microbial interactions.

The direct application of systematics to practical agriculture is more subtle. Quantitative relationships among intraspecific populations, among species, and even among higher-level taxa often inform about biological characteristics associated with benefit, risk, and methods of control. For example, an understanding of the intraspecific phylogeny of an invasive weed can help determine its native point of origin, helping to target efforts to find biological control agents.

The information accumulated by ARS is best exemplified by the major collections it maintains. The major arthropod collections are part of the U.S. National Museum and consist of millions of individual specimens (www.ars.usda.gov/main/site_main.htm?modecode=12-45-49-00). The three ARS Overseas Biological Control Laboratories maintain plant and arthropod collections for their own reference and documentation, although they also actively deposit voucher specimens in museums. Collections of pathogens that kill insects are maintained at several locations, with the main one located at the Biological Integrated Pest Management Research Unit in Ithaca, New

York. Outside of NP 304, the U.S. National Fungus Collections in Beltsville, Maryland, are a major national resource for the country. Live collections include the National Plant Germplasm System (www.ars-grin.gov/npgs/index.html), the National Microbial Germplasm Program (www.ars-grin.gov/nmg/index.html), the National Animal Germplasm Program (http://nrrc.ars.usda.gov/A-GRIN/main_webpage/ars?record_source=US), and the National Invertebrate Genetic Resources Program (www.ars-grin.gov/nigrp/index.html). This system of collections is an example of how ARS is able to connect different research directions to encourage interdisciplinary innovation. NP 304 funds only some of the collections described, but they all interact within the Agency for the benefit of systematic research.

The NP 304 Action Plan includes three Problem Statements that were expected to guide the 5-year research plan and the development of the anticipated products in this Component. The Problem Statements and the ARS research accomplishments that address each of them are presented below.

PROBLEM STATEMENT IA: *Insects and Mites*

Arthropods are particularly varied and numerous, constituting more different species than all other organisms combined. The significance of this characteristic to agriculture can hardly be overstated. Agriculture depends on arthropods for ecological systems services such as pollination, pest management, and soil conditioning. Crops in the field are constantly challenged by herbivorous arthropods, which by some estimates cause an overall average crop loss in the United States of 50 percent if uncontrolled, and even under controlled conditions they continue to cause 13 percent loss ($26 billion a year). Integrated pest management (IPM) is a system that attempts to control pests economically while preserving the environment, and it includes the use of beneficial insects. The “integrated” in IPM demands considerable technical expertise and background knowledge. One of the principal requirements is knowledge of the kinds of arthropods that cause damage or are beneficial.

ARS is a national resource for arthropod taxonomy. Efforts range from basic science resulting from systematics studies to application of better methods for identification. During the last 5 years, ARS has accomplished major revisions of significant groups, described species in support of biological control programs, and helped APHIS produce tools for detection of pests that could invade the United States. Some of the work involves population genetics applied to distinguish genetic structure and gene flow within species.

Hidden Neotropical diversity. Groups of plant-feeding insects may include suites of species that range from highly adapted specialists that feed on a single species of plant to generalists that feed on many different kinds of plants. Cryptic species are often included in these insect groups, making precise identification impossible using morphological methods and obscuring true ecological relationships of the plants and the insects. ARS scientists in Beltsville, Maryland, were part of a team that made a significant contribution to biological theory by using molecular methods to identify insects, as described in an article in *Science* (cited below). The team used molecular techniques to assess the number of species of *Blepharoneura* tephritid flies in cucurbit flowers occurring in 10 biogeographic zones throughout Latin America, from Mexico to Bolivia.

Sampling 419 plants in 9 genera, the investigators identified 52 genetically defined species based on the conservative criterion of a 4-percent sequence difference in the cytochrome c oxidase subunit 1 mitochondrial gene. Contrary to expectations, the team found a variety of evolutionary patterns for this genus resulting in different levels of biodiversity regionally and within species of plants. For example, rare plant species were not hosts to generalist species of the flies. Also, there were locally endemic species on continentally widespread host plants, suggesting that there has been a history of habitat fragmentation during the estimated 2.6 million years leading to the speciation patterns observed. Most significantly, the team found multiple species of flies in the same plant part, which was a clear contradiction to the usual assumption of niche partitioning among closely related species. This was a basic discovery, but it informs practical efforts in biological control by broadening the range of ecological situations that should be explored for natural enemies. The findings also imply that among invasive species there may be a complex pattern of evolutionary adaptation after introduction, suggesting an increased need to study potential ecological roles of introduced species.

Condon, M., Scheffer, S.J., Lewis, M.L., and Swensen S. 2008. Hidden Neotropical diversity: greater than the sum of its parts. Science 320:928–931.

First molecular phylogeny of the family Tortricidae (leaf-roller moths). Phylogenies provide working hypotheses of genealogical relationships among organisms that can be used to develop more robust and meaningful classifications, track the evolution of character states, and potentially predict behavior, host utilization, and invasiveness of plant-feeding insects. ARS scientists, working with international collaborators that included scientists from the University of Maryland and Smithsonian Institution and funded in part by an NSF grant, "Discovering the Tree-of-Life," developed the first molecular phylogeny for the leaf-roller moth family Tortricidae, which includes numerous pests of crops, ornamentals, and forests worldwide. Feeding habits (internal versus external) were shown to be highly compatible with the phylogeny, with fruit feeding and seed feeding restricted to specific pest groups. Within a large group characterized by eggs being deposited in large clusters, as opposed to singly or in small batches, there is a conspicuously higher proportion of polyphagous species (those that attack a wide range of plant families). Virtually all of the latter are external feeders and are considerably easier to detect through visual inspection compared to internal fruit feeders and seed feeders, the latter of which cause direct damage to high-value fruit commodities. Therefore, tortricids from other countries that deposit single eggs or small clusters of eggs are more likely to be important invasive pests, justifying greater efforts to detect them on imported plants or fruits. These findings will provide a broad-scale framework for predicting which tortricid species are most likely to cause significant economic damage to fruit and seed crops and which species may elude detection at U.S. ports of entry.

Regier, J. C., Mitter, C., Zwick, A., Bazinet, A.L., Cummings, M.P., Kawahara, A., Sohn, J.-C. Zwickl, J.D., Cho, S., Davis, D.R., Baixeras, J., Brown, J., Parr, C., Weller, S., and Lees, D. 2013. A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). PLoSOne 8(3):e58568 (23 pages).

Regier, J., Brown, J., Mitter, C., Baixeras, J., Cho, S., Cummings, M., and Zwick, A. 2012. A molecular phylogeny for the leaf-roller moths (Lepidoptera: Tortricidae) and its implications for classification and life history evolution. PLoS One 7(4):e35574 (17 pages).

Cho, S., Zwick, A., Regier, J., Mitter, C., Cummings, M., Yao, J., Du, Z., Zhao, H., Kawahara, A., Weller, S., Davis, D., Baixeras, J., Brown, J., and Parr, C. 2011. Can deliberately incomplete gene sample augmentation improve a phylogeny estimation for the advanced moths and butterflies (Hexapoda: Lepidoptera)? Systematic Biology 60:782–796.

Regier, J., Zwick, A., Cummings, M., Kawahara, A., Cho, S., Weller, S., Roe, A., Baixeras, J., Brown, J., Parr, C., Davis, D., Epstein, M., Hallwachs, W., Hausmann, A., Janzen, D., Kitching, I., Solis, A., Yen, S.-H., Bazinet, A., and Mitter, C. 2009. Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): initial molecular study. BMC Evolutionary Biology 9:280, 21 pp.

Manual of the flies (Diptera) of Central America. The order Diptera (flies)—the second-largest order of insects after beetles—includes important key pests for agriculture, such as fruit flies (Tephritidae), gall gnats (Cecidomyiidae), and leaf-mining flies (Agromyzidae). The order also includes predatory and parasitic species that are part of the complex of insects that are essential for natural regulation of insect populations. The tens of thousands of Diptera species present many challenges for identification and classification. ARS scientists in Beltsville, Maryland, contributed to, and helped edit, *Manual of Central American Diptera*, a comprehensive identification manual for the flies of Neotropical Mexico and Central America. This was a large-scale, collaborative project involving many of the world's leading fly taxonomists, including three from ARS. The two-volume book of nearly 1,500 pages provides identification keys to the generic level, copious illustrations, and chapters on biology, economic importance, and evolutionary relationships of flies. Most of the chapters involved significant original research because very few identification tools for any groups of flies existed for the fauna of southern Mexico and Central America. ARS scientists helped to develop the information or were principal sources for information on species in 22 chapters of the two volumes. This work is now the fundamental reference for flies from southern Mexico and Central America. The practical impact of this work is that it will facilitate accurate identifications for faunal surveyors, producers with pestiferous Diptera, and scientists working on biological control of pests.

Brown, B.V., Borkent, A., Cumming, J.M., Wood, D.M., Woodley, N.E., and Zumbado, M.A. (eds.). 2010. Manual of Central American Diptera. Volume 2. NRC Research Press, Ottawa i-xvi + 715–1442.

Brown, B.V., Borkent, A., Cumming, J.M., Wood, D.M., Woodley, N.E., and Zumbado, M.A. (eds.). 2009. Manual of Central American Diptera. Volume 1. NRC Research Press, Ottawa i-xii + 1–714.

Identification tools assist in the early detection and exclusion of fruit fly pests. While there are many species of Tephritid fruit flies, relatively few become agricultural pests. However, those that do are key pests that often damage high-value fruit crops. These species must be identified in order to control them where they exist and to prevent them from entering new areas. ARS scientists in Beltsville, Maryland, developed new identification tools for tephritid fruit flies of the genus *Anastrepha*, the largest and most economically important group of fruit flies in the American tropics. The flies in this group attack a wide range of commercial crops, such as citrus, mango, peach, and apple. This work included the discovery, description, and illustration of 25 previously unknown species, as well as detailed descriptions and illustrations of more than 200 others. Information for the more than 250 species of this group is being made available via an online electronic identification tool. This tool is being used by APHIS-Plant Protection and

Quarantine (PPQ) and other regulatory agencies to identify flies in this group and prevent the introduction of pest species during the importation of fruit from overseas.

Norrbom, A.L. and Korytkowski, C.A. 2012. A new species of *Anastrepha* (Diptera: Tephritidae) from Colombia, Costa Rica and Panama. Canadian Entomologist 144:158–168.

Norrbom, A.L. and Korytkowski, C.A. 2012. New species of *Anastrepha* (Diptera: Tephritidae), with a key for the species of the *megacantha* clade. Zootaxa 3478:510–552.

Norrbom, A.L. and Korytkowski, C.A. 2011. New species of and taxonomic notes on *Anastrepha* (Diptera: Tephritidae). Zootaxa 2740:1–23.

Norrbom, A.L. and Uchôa, M.A. 2011. New species and records of *Anastrepha* (Diptera: Tephritidae) from Brazil. Zootaxa 2835:61–67.

Novel tools help to identify potential invasive longhorned woodboring beetles from Hispaniola. There is an urgent need to know the insect fauna of the Caribbean region because it is an important and nearby source of invasive beetles into the United States. Based on studies of the longhorned beetles (Coleoptera: Cerambycidae) of Hispaniola, ARS scientists in Washington, D.C., have developed tools crucial for identifications performed by APHIS-PPQ and State agricultural extension agencies in their efforts to recognize and prevent new introductions. The publications from this work (three of which are cited below) describe 40 percent more species than were known before. Thirty-five previously unknown species have been described, and new country distributional records have been established for 20 more species. The researchers developed new identification tools (including dichotomous keys, color illustrations, and microphotographs) for four tribes and eight genera of longhorned beetles and have disseminated the information to port identifiers (APHIS-PPQ), agricultural extension agencies, and researchers as part of the combined effort to protect U.S. agriculture from potentially harmful invasive beetle pests.

Lingafelter, S.W. 2013. Hispaniolan Hemilophini (Coleoptera, Cerambycidae, Lamiinae). ZooKeys 258:53–83.

Lingafelter, S.W. 2010. Methiini and Oemini (Coleoptera: Cerambycidae: Cerambycinae) of Hispaniola. The Coleopterists Bulletin 64(3):265–269.

Lingafelter, S.W. and Micheli, C.J. 2009. The genus *Leptostylopsis* of Hispaniola (Coleoptera: Cerambycidae: Acanthocinini). ZooKeys 217(17):1–55.

***New tools developed for the identification of the beetle genus* Diabrotica.** *Diabrotica* is one of the most economically important leaf beetle genera, with a single species, *D. virgifera*, costing the U.S. economy about $1 billion annually. Many *Diabrotica* species have been intercepted at U.S. ports of entry every year, but they could not be identified because of a lack of modern treatments and identification keys. After 2 years of extensive study, ARS scientists in Washington, D.C., in collaboration with APHIS-PPQ and the University of Maryland Department of Entomology, documented all 123 *Diabrotica* species historically known to occur in North and Central America and prepared an illustrated, interactive key to the species. The researchers discovered 11 new synonyms and 22 new species and reevaluated the status of several species. The researchers have launched a Web site that provides all available information (http://idtools.org/id/beetles/diabrotica/index.php) and includes an interactive key

for all 112 valid *Diabrotica* species in North and Central America. This makes it possible to accurately and rapidly identify to species level *Diabrotica* specimens intercepted at U.S. ports of entry, thus protecting U.S. agriculture from potentially invasive and devastating *Diabrotica* pests from Central America.

Derunkov, A., Konstantinov, A., Tishechkin, A., Hartje, L., and Redford, A.J. 2013. Diabrotica ID: Identification of Diabrotica species (Coleoptera: Chrysomelidae) from North and Central America. USDA APHIS PPQ Center for Plant Health Science and Technology, USDA Agricultural Research Service, University of Maryland, and Louisiana State University http://idtools.org/id/beetles/diabrotica/

Derunkov, A. and Konstantinov, A. 2013. Taxonomic changes in the genus *Diabrotica* Chevrolat (Coleoptera: Chrysomelidae: Galerucinae): results of a synopsis of North and Central America *Diabrotica* species. Zootaxa 3686(3):301-325. http://dx.doi.org/10.11646/zootaxa.3686.3.1

Thirty-four new insect species described in Argentina. The process of finding and developing biological control agents is a complicated procedure in applied ecology that can provide not only permanent and safe solutions to invasive species problems, but also many details about the biology of insects and plants. During those detailed studies, it is essential to be able to identify both targets and potential biological control agents accurately. In many cases, the insect-plant system has never been studied in detail, so it is not surprising when completely new species are discovered. During extensive field explorations over the last 5 years, ARS scientists at the Foundation for the Study of Invasive Insects, FuEDEI (formerly the South American Biological Control Laboratory) in Hurlingham, Buenos Aires Province, Argentina, discovered 34 new species of insects. Twenty-three of the species were named by expert taxonomists in close collaboration with FuEDEI scientists. The descriptions of 11 species are in progress by FuEDEI and its associates in Argentina. This accomplishment greatly enhances the success of respective biological control programs in the United States and the knowledge of the biological diversity in Argentina and globally. The complete list of species and publications is available at www.fuedei.org. Two representative publications are cited below.

Martinez, J.J., Berta, C., Varone, L., Logarzo, G.A., Zamudio, P., Zaldivar-Riveron, A., and Aguilar- Velazco, R.G. 2012. DNA barcoding and morphological identification of Argentine species of *Apanteles* (Hymenoptera: Braconidae) parasitoids of cactus feeding moths (Lepidoptera: Pyralidae: Phycitinae), with description of a new species. Invertebrate Systematics 26:435–444.

Triapitsyn, S.V. and Hernández, M.C. 2011. 'Egg parasitoids of *Taosa* spp. (Hemiptera: Dictyopharidae) in Formosa, Argentina, with descriptions of two new species', Revista de la Sociedad Entomológica Argentina, 70(1-2):55-61.

New hope for endangered cycad forests and urban plantings of king sago palms. A tiny insect known as the cycad aulacaspis scale has spread from Thailand into many warm regions of the world, where it poses a threat to wild cycad forests and ornamental plantings of king sago palms. Cycads, such as the king sago palm, are highly valued in the United States and other countries for their beauty and functionality as landscape and container plants and as an important food source for peoples in the Caribbean, Mexico, Asia, and Africa. ARS scientists in Beltsville, Maryland, and a collaborator from Universidadae Federal do Para in Brazil described and named a new beneficial species of lady beetle that is being reared for release against the scale pest.

Biological control researchers at the University of Florida and the Florida Department of Agriculture and Consumer Services discovered the new lady beetle during biological control expeditions to Thailand in search of natural enemies that feed on the scale. The publication (cited below) describing the new lady beetle species will help facilitate the necessary Federal approvals to make local releases of what appears to be a very promising and dedicated predator of cycad aulacaspis scale.

Giorgi, J.A. and Vandenberg, N.J. 2012. Review of the lady beetle genus *Phaenochilus* Weise (Coleoptera: Coccinellidae: Chilocorini) with description of a new species from Thailand that preys on cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Hemiptera: Sternorrhyncha: Diaspididae). Zootaxa 3478:239–255.

New potential biological control agent against Brazilian peppertree leads to discovery of five additional new species. The Brazilian peppertree is one of the most damaging invasive weed species in Florida. Introduced as an ornamental at least three times in the mid- to late-1800s, it has since invaded a variety of habitats throughout the central and southern parts of the State. During investigations focused on the biological control of the Brazilian peppertree, University of Florida researchers in Fort Pierce, Florida, sent specimens of a small moth to ARS scientists in Beltsville, Maryland, for identification. Although the moths were initially identified as *Paectes obrotunda* (Guenée), further examination revealed that they represent a species new to science. The scientists also determined that specimens previously identified as *P. obrotunda* actually represented three valid species and five species new to science. All species have very similar forewing color and pattern, making precise determinations difficult; however, characters in the male and female genitalia were discovered to be distinct among the species. A revision of the group also revealed that two additional species of *Paectes* use Brazilian peppertree as host plants—one is native to Florida and the other is widespread across South America. This research will be valuable to scientists working on the biological control of this exotic plant.

Pogue, M.G. 2013. A review of the *Paectes arcigera* species complex (Guenée) (Lepidoptera, Euteliidae). ZooKeys 264:125–163.

Manrique V., Diaz, R., Pogue, M.G., Vitorino, M.D., and Overholt, W.A. 2012. Description and biology of *Paectes longiformis* (Lepidoptera: Euteliidae), a new species from Brazil and potential biological control agent on Brazilian peppertree in Florida. Biocontrol Science and Technology 22:163–185.

Molecular taxonomic research supports use of dung beetle as a biological control agent. The introduction of cattle and sheep into Australia and the intensification of rearing have led to high levels of dung accumulation in pastures and a corresponding proliferation of coprophilous and hematophagous flies. The introduction of exotic dung beetles in the 1960s significantly ameliorated the situation, but further improvements were desired. After exhaustive scientific study, Australian regulators issued an importation permit for release of a Eurasian species of dung beetle. However, the project was delayed by questions that arose about the taxonomic status of the beetles collected in Europe for export to Australia. The ARS European Biological Control Laboratory in Montpellier, France, was asked to join a team of French, Australian, and New Zealand researchers to determine the exact taxonomic status of the dung beetle through molecular genetics sequencing as a precondition for its introduction into Australia. To investigate the taxonomic status, the ARS scientists and their collaborators sequenced several

gene regions present in a range of morphological types that are representative of the diversity of dung beetle populations in southern France, the native range of the dung beetle. Individual gene markers gave contradictory results. The research results suggested that two evolutionary lineages are found in French populations of the dung beetle, demonstrating the importance of using several different markers when attempting to determine species limits. The results allowed the initial introductions of certain populations of this beetle into Australia to proceed with the release program as planned and facilitated the continuation of Australia's biological control program. The results are expected to benefit a similar project in New Zealand.

Boullis, A., Kirk, A., Serin, J., Kadiri, N., Lumaret, J.P., and Bon, M.C. 2013. Integrating morphological and molecular approaches for characterizing *Onthophagus vacca* and *O. medius* (Coleoptera: Scarabaeidae) as an import compliance procedure for Australia. Bulletin of Entomological Research in press.

Invasive vine mealybug in California originated in the Middle East. The invasive vine mealybug has become an important pest of grapevines in California. Field collections of the invasive vine mealybug in its native range by ARS scientists at the European Biological Control Laboratory in Montpellier, France, contributed to a study of genetic diversity of native range populations compared with those in introduced regions. More than 50 populations in eight European countries were collected and shipped by ARS to a close collaborator at the University of California, Berkeley. The populations were analyzed at Berkeley and compared to specimens from 52 invasive vine mealybug populations that had been collected in vineyards from North and South America, Europe, Africa, and the Middle East. Data from the mitochondrial cytochrome oxidase I gene and the nuclear ITS-1 region showed that vine mealybugs in North America are from the Middle East, with California populations likely coming from Israel. This is the first molecular phylogeographic study of invasive vine mealybug showing structure among the populations and inferring invasion history. Other populations in Argentina and South Africa are derived from European sources. A high degree of molecular variation divided the mealybug samples into two broad groupings, suggesting the possibility of a cryptic species. Systematics studies have also been important in using a parasitoid, *Anagyrus* sp., against the invasive vine mealybug in California. Working with the University of California, Berkeley, ARS scientists showed that *Anagyrus pseudococci* comprises two cryptic species. Mass releases of this parasite have already been carried out, but knowledge of the species complex will allow more precise targeting of the right species in the right area of California.

Daane, K.M., Almeida, R.P.P., Bell, V.A., Walker, J.T.S., Botton, M., Fallahzadeh, M., Mani, M., Miano, J.L., Sforza, R., Walton, V.M., and Zaviezo, T. 2012. Biology and management of mealybugs in vineyards, pp. 271-307. In N. J. Bostanian, R. Isaacs, and C. Vincent (eds.) Arthropod Management in Vineyards. Springer, The Netherlands 505pp.

Daane, K.M., Middleton, M.C., Sforza, R., Cooper, M.L., Walton, V.M., Walsh, D.B., Zaviezo, T., and Almeida, R.P.P. 2011. Development of a multiplex PCR for identification of vineyard mealybugs. Environmental Entomology 40(6):1595-1603.

Systematic study of the tomato fruit borer for quarantine purposes. The tomato fruit borer (Lepidoptera: Crambidae: *Neoleucinodes elegantalis*), the most devastating pest of tomatoes in South America, does not yet occur in the United States, but it has associated quarantine restrictions that affect the importation into the United States of a wide variety of solanaceous

vegetables, including peppers and tomatoes. Collaborative research between ARS scientists in Beltsville, Maryland, and APHIS scientists in Riverdale, Maryland, resulted in the development of new tools for the identification of various life stages of the tomato fruit borer and related species, the compilation of their known hosts in Colombia and elsewhere, and a review of current control measures for the species in South America. These studies of where this key pest is found, how it is identified, and what plants it attacks have direct practical benefit for the purpose of excluding it from the United States. Inspectors will be able to identify the pest accurately, concentrate their inspection efforts on the right plants, and appreciate the importance of keeping it out. This research provides information critical for U.S. quarantine personnel to detect and exclude this species from imported solanaceous vegetables. This information was integrated into the latest USDA APHIS CPHST Pest Datasheet (http://caps.ceris.purdue.edu/webfm_send/1853).

Diaz Montilla, A.E., Solis, M.A., and Kondo, T. 2013. The fruit borer, *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae), an insect pest of neotropical solanaceous fruits, *In* J Peña (Ed.), Potential Invasive Pests of Agricultural Crops, CABI, London.

Diaz, A.E., Solis, M.A., and Brochero, H.L. 2011. Distribución geográfica de *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) en Colombia. Revista Colombiana de Entomologia 37(1):73-78.

Diaz, A.E. and Solis, M.A. 2007. A new species and species distribution records of *Neoleucinodes* (Lepidoptera: Crambidae: Spilomelinae) from Colombia feeding on *Solanum* spp. Proceedings of the Entomological Society of Washington 109(4):897-908.

Methods for reliably separating adult apple maggot and snowberry maggot flies identified. To decrease the risk of unnecessary apple maggot quarantines, methods are needed to reliably discriminate between the apple maggot fly (an apple pest) and the snowberry maggot fly (a non-pest). The sibling species are nearly identical morphologically and genetically, with no apparent diagnostic differences between them. ARS scientists in Wapato, Washington, and their collaborators at Canisius College in Buffalo, New York, used geometric morphometric techniques along with traditional morphometric methods to show that morphological traits combined with body measurements of the two fly species can reliably separate and identify the species with greater than 90-percent accuracy. The information has been disseminated by ARS to industry and other scientists through conference presentations, grower meetings, and publications. The methods developed in this research have been used to help the Washington State Department of Agriculture with its apple maggot fly detection program and has resulted in cost savings for the State and for apple growers.

Yee, W.L., Chapman, P.S., and Sheets, H.D. 2013. Body size and shape analyses of F_1 hybrid *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae). Annals of the Entomological Society of America 106(4):410-423. www.bioone.org/doi/full/10.1603/AN13016

Yee, W.L., Sheets, H.D., and Chapman, P.S. 2011. Analysis of surstylus and aculeus shape and size using geometric morphometrics to discriminate *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae). Annals of the Entomological Society of America 104(2):105–114.

Yee, W.L., Chapman, P.S., Sheets, H.D., and Unruh, T. R. 2009. Analysis of body measurements and wing shape to discriminate *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae) in Washington state. Annals of the Entomological Society of America 102(6):1013–1028.

PROBLEM STATEMENT IB: *Weeds*

Noxious weeds inhabit over 100 million acres in the United States and are increasing their range by as much as 8–20 percent each year. The economic impact of weeds is estimated to be nearly $30 billion per year. Some of those weeds compete directly with crop species, obliging farmers to either spend resources on weed control or suffer losses in yield. Other weeds have invaded natural landscapes, threatening native plant species, increasing the risk of fire, and decreasing wildlife habitat. The use of systematics to combat noxious weeds is important for several reasons. First, identification of weeds is essential to effective control because management techniques will vary depending on the type of weed. Second, crossing between species and biotypes is common, sometimes creating new qualities in the weeds that make them even harder to manage. Finally, accurate phylogenies analyzed by geographic vicariance can show the native origin of species, sometimes leading to the discovery of effective biological control agents. Weed systematics within ARS has been directed at specific problems, and its accomplishments support applied programs for biological control of weeds affecting natural ecosystems, pastures, and crops. One area of emphasis is tracing the geographic origin of invasive weeds to be able to target efforts to find natural enemies and to identify genetic recombinations that impart new characteristics on invasive weeds.

Scholarly reviews of molecular methods for biological control of weeds. When seeking control of a target weed, accurate identification can prevent expensive development of biological control against the wrong weed. ARS scientists in Sidney, Montana, Fargo, North Dakota, and Montpellier, France, worked with CABI (a private international foundation for the promotion of agriculture) and the Australian Commonwealth Scientific and Industrial Research Organisation to produce reviews of molecular methods as an aid in the battle against invasive weeds. These methods are helpful in a number of aspects of invasive weeds study. First, molecular review using sequencing or amplified fragment length polymorphisms can help distinguish whether a previously identified species is actually more than one species. Second, detailed genetic analysis can determine which populations within a species are hybridizing. One of the problems following establishment of an invasive weed is that genetically varied, introduced populations may hybridize in ways that do not occur in the native range of the plant. This can produce weeds that have entirely different properties, often with deleterious consequences for the invaded range. Knowing that such hybridization has occurred informs developers of biological controls that they should test natural enemies against relevant biotypes. Finally, phylogenetic analysis of genetic sequences can lead to the geographic origin of a weed and the most likely place to search for biological control agents. This work advances the sophistication of biological weed control, potentially saving years in the development of a biological control agent.

Gaskin, J.F., Bon, M.C., Cock, M.J.W., Cristofaro, M., De Biase, A., De Clerck-Floate, R., Ellison, C.A., Hinz, H., Hufbauer, R., Julien, M., and Sforza, R. 2011. Applying molecular-based approaches to classical biological control of weeds. Biological Control 58:1–21.

Horvath, D.P. 2010. Genomics for Weed Science. Current Genomics 11:47-51(5).

Distinguishing characteristics of southeastern prickly nightshades identified. Prickly nightshades are troublesome weeds of natural habitats, pastures, feedlots, right-of-ways, and croplands. ARS scientists in Stoneville, Mississippi, compared native and nonnative invasive weedy species of prickly nightshades to determine growth, development, and morphological differences. They then developed databases from new and existing data to differentiate vegetative and reproductive characteristics among 18 species of prickly nightshade found in the southeastern United States. Six of the 18 species studied (*Solanum bahamense, S. capsicoides, S. carolinense, S. dimidiatum, S. donianum, and S. pumilum*) are native to the United States. Two species (*S. citrullifolium* and *S. rostratum)* are annuals; the others are perennials or short-lived perennials or annuals in northern extremes of their range in North America. These biological and morphological data can be used to help determine the weeds' most vulnerable stage in order to develop the most effective control methods and strategies. Such taxonomic work is relied on as the basis for more accurate identification in local guides that are routinely used to identify invasive weeds.

Bryson, C.T., Reddy, K.N., and Byrd, Jr. J.D. 2012. Growth, development and morphological differences among native and nonnative prickly nightshades (*Solanum* spp.*)* of the Southeastern United States. Invasive Plant Science and Management 5:341-352.

Population genetics of Canada thistle. Canada thistle is a noxious perennial weed that invades natural ecosystems and agroecosystems. Efforts to develop biological control agents have been challenged by the difficulty of showing that these agents will not affect valued native species of thistles. ARS scientists in Fargo, North Dakota, and Montpellier, France, together with university partners in British Columbia, Canada, collected populations of thistles from across North America, Europe, and parts of Asia for analysis. The researchers developed molecular markers to examine the genetic diversity and phylogeny of Canada thistle and native thistles in the United States. They showed that insect biological control agents do not attack the phylogenetic clade containing North American thistle species. The study also established the invasion pathways of Canada thistle from Eurasia to the United States. This research provides a comprehensive framework to rationally evaluate the potential for biological control of Canada thistle and targets the Eurasian region for exploration.

Bodo Slotta, T.A., Horvath, D.P., and Foley, M.E. 2013. Relationships within *Cirsium* spp.: Implications for biological control. Plants 1:61-73.

Guggisberg, A., Welk, E., Sforza, R., Horvath, D.P., Anderson, J.V., Foley, M.E., and Rieseberg, L.H. 2012. Invasion history of North American Canada thistle, *Cirsium arvense*. Journal of Biogeography 39:1919-1931.

Bodo Slotta, T.A., Foley, M.E., Chao, S., Hufbauer, R., and Horvath, D.P. 2010. Assessing genetic diversity of Canada thistle (*Cirsium arvense*) in North America with microsatellites. Weed Science 58:387–394.

Major invasive plant is actually a hybrid species. Saltcedar trees (*Tamarix ramosissima* and *T. chinensis*) are native to Eurasia but have become common and invasive in many western U.S. river habitats since their intentional introduction. An initial analysis of DNA by ARS scientists in Sidney, Montana, showed that about a quarter of the plants in the United States were a novel

hybrid of these two species that does not occur in the native ranges of the plants. Further DNA work by the scientists determined that up to 87 percent of the weeds in the invaded range are hybrid. The findings cause concerns that the high percentage of novel hybrids may limit the potential of classic biological methods to provide effective control. Hybrids have also been shown to adapt better to the varied climates in the United States. As a result of this work, researchers developing biological control agents against this weed know they will need to test effectiveness against a range of biotypes.

Gaskin, J.F. 2012. Tamaricaceae. In: Baldwin, B.G., Goldman, D.H., Keil, DJ, Patterson, R., Rosatti, T.J., Wilken, Dieter, D.H., editors. The Jepson Manual: Vascular Plants of California, Second Edition. Berkeley, CA: University of California Press pp.1259-1261.

Gaskin, J.F. and Kazmer, D.J. 2009. Introgression between saltcedars (*Tamarix chinensis* and *T. ramosissima*) in the USA invasion. Biological Invasions 11:1121-1130.

Friedman, J.M., Rolle, J.E., Gaskin, J.F., Pepper, A.E., and Manhart, J.R. 2008. Latitudinal variation in cold hardiness in introduced *Tamarix* and native *Populus*. Evolutionary Applications 1:598-607.

PROBLEM STATEMENT IC: *Microbials*

Beneficial microbial agents can be important biological control agents of insects and weeds, and identification and maintenance of different strains is essential to their development and use. There are many sources of bacteria, fungi, and nematodes that might be effective and safe insect as weed control agents, which creates a challenge for identification and classification. Modern molecular techniques have made the development of phylogenies that can be helpful in predicting the qualities of new agents routine. Considering the extra scrutiny these agents undergo before release, predictive tools are very helpful.

NP 304 scientists performed microbial systematics in support of weed and insect control. Systematic examination of microbial collections identified new candidate insect control agents. Research efforts have also included exploration for microbial biocontrol agents. That exploration involved culture, identification, and bioassay.

Culture collection a globally essential resource for research on fungi from invertebrates.
Insects are subject to pathogens that, under some circumstances, cause population level reductions. Fungi include many insect pathogens, and their characterization presents a major challenge because of their hidden life stages, subtle differences in species, and ability to hybridize widely. The USDA ARS Collection of Entomopathogenic Fungal Cultures in Ithaca, New York, continues to be the pre-eminent American resource of fungal germplasm originating from insects, mites, ticks, and other invertebrates. The collection now includes nearly 12,000 isolates of some 700 fungal taxa that were collected from 1,300 host arthropods or 2,350 substrates from around the world. During the last 5 years, ARS has accessioned nearly 3,200 new isolates and provided 2,800 fungi to more than 220 individual recipients. ARS has been a key research partner in a number of phylogenetically based revisions and overviews of fungi, including *Beauveria, Metarhizium*, Entomophthorales, and insect-associated isolates of *Fusarium*. One of the significant taxonomic actions performed by the scientists was the

reclassification of the class Entomophthorales to the phylum Entomophthoromycota, which now includes the classes Basidiobolomycetes, Neozygitomycetes, and Entomophthoromycetes. At a more specific taxonomic level, ARS scientists in Ithaca and Weslaco, Texas, described *Isaria poprawskii* as a new species that tolerates high temperatures and is pathogenic to whiteflies and other pests of the Lower Rio Grande Valley. As the central repository for entomopathogenic fungi, this collection provides a resource for organisms and genomes that affect all insects and that might eventually be used as biopesticides.

Gryganskyi, A.P., Humber, R.A., Smith, M.E., Hodge, K., Huang, B., Voigt, K., and Vilgalys, R. 2013. Phylogenetic lineages in *Entomophthoromycota*. Persoonia 30:94-105.

Gryganskyi, A.P., Humber, R.A., Smith, M.E., Miadlikovska, J., Wu, S., Voigt, K., Walter, G., Anishchenko,I.M., and Vilgalys, R. 2012. Molecular phylogeny of Entomophthoromycota. Molecular Phylogenetics and Evolution 65:682-694.

Humber, R.A. 2012. Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. Mycotaxon 120:477-492.

O'Donnell, K., Humber, R.A., Geiser, D.M., Kang, S., Park, B., Robert, V.A.R.G., Crous, P.W., Johnston, P.R., Aoki, T., and Rehner, S.A. 2012. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via the Internet at FUSARIUM-ID and *FUSARIUM MLST.* Mycologia 104:427-445.

Rehner, S.A., Minnis, A.M., Sung, G.-H., Luangsa-ard, J.J., Devotto, L., and Humber, R.A. 2011. Phylogenetic systematics of the anamorphic, entomopathogenic genus *Beauveria* (Hypocreales, Ascomycota). Mycologia 103:1055-1073.

Integration of molecular taxonomy and biological control. Determining host range of classic biological control agents has been a cumbersome process, often involving tests of 50 or more non-target species under conditions that make it difficult to predict results in the field. ARS scientists in Fort Detrick, Maryland, in collaboration with Russian scientists and researchers at the ARS laboratory in Thessaloniki, Greece, developed a procedure to generate best linear unbiased predictors based on disease reaction and host DNA sequences to determine the probable host range in the field of plant pathogens for classic biological control of weeds. The researchers applied molecular methods for classification and evaluation to discover new biological control agents against yellow starthistle (*Puccinia jaceae*), Canada thistle (*Puccinia punctiformis*), and Russian thistle or tumbleweed (*Colletotrichum gloeosporioides* and *Uromyces salsolae*). Biological control will aid agriculture and return natural habitats to a more balanced state. A potential impact is more accurate and safer evaluation of host range than can be obtained with other methods and fewer non-target effects once the agent is released in the field.

Berner, D.K. 2010. BLUP, a new paradigm in host-range determination. Biological Control 53:143-152.

Berner, D.K., Bruckart, W.L., Cavin, C.A., and Michael, J.L. 2009. Mixed model analysis combining disease ratings and DNA sequences to determine host range of Uromyces salsolae for biological control of Russian thistle. Biological Control 49:68-76.

Woods, D.M., Bruckart, W.L., Pitcairn, M., Popescu, V., and O'Brien, J. 2009. Susceptibility of yellow starthistle to Puccinia jaceae var. solstitialis and greenhouse production of inoculum for classical biological control programs. Biological Control 50:275-280.

Kolomiets, T., Skatenok, O., Alexandrova, A., Mukhina, Z., Matveeva, T., Bogomaz, D., Berner, D.K., and Cavin, C.A. 2008. First report of anthracnose of Salsola tragus caused by Colletotrichum gloeosporioides in Russia. Plant Disease 92:1366.

Phylogenetics of entomopathogenic fungi. *Beauveria* is a cosmopolitan genus of arthropod pathogens that includes the agronomically important species, *B. bassiana* and *B. brongniartii*. *B. bassiana*, in particular, is commonly used as a biopesticide and is the subject of study by ARS as an alternative to synthetic chemical pesticides. ARS scientists in Beltsville, Maryland, performed a phylogenetic analysis of the entire genus and sister groups to show that *Beauveria* is monophyletic, meaning that all members of the genus share a common evolutionary ancestor. The study was the first taxonomic review of the genus in over 30 years. Using sequence data from several genes to compare similarity and divergence of species, the revisionary work established new type specimens and six new species. This work supports applied studies that examine members of the genus *Beauveria* for insecticidal potential in that membership in the genus is now known to be evolutionarily significant.

Rehner, S.A., Minnis, A.M., Sung, G.H., Luangsa-ard, J.J., Devotto, L., and Humber, R.A. 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus Beauveria. Mycologia 103(5):1055-1073.

Practical results from ARS worldwide collection of diverse Bacillus thuringiensis isolates. *Bacillus thuringiensis* (Bt) is an important bacteria used for safe control of many kinds of insects. Strains of Bt are specific for insect groups and have different properties. ARS scientists in Beltsville, Maryland, have assembled a collection of more than 40,000 strains that form a repository for these important bacteria, as well as a resource for further study. The scientists discovered that many strains of Bt are capable of killing gypsy moth larvae; however, only those strains that produce the enzyme urease are capable of surviving as true pathogens. Bt strains that lacked the ability to produce urease were not capable of surviving repeated passages through gypsy moth larvae, whereas virtually all urease-positive strains tested were capable of repeated passage. Phenotypic analysis of 3,639 Bt isolates from around the world revealed that urease production was highly correlated with production of bipyramidal crystals and lepidopteran toxicity; phylogenetic analysis showed that the vast majority of urease-producing Bts belonged to the varieties *kurstaki* or *toumanoffi*. The scientists also found that Bt varieties *morrisoni* and *toumanoffi* can share common plasmid-bearing genes for insecticidal protein toxins previously unknown in Bt. These toxins were known previously only from gram-negative genera of insect pathogenic bacteria such as *Photorhabdus* and *Yersinia*. The toxin genes were expressed during pathogenesis in gypsy moth. Screening for urease activity allows identification of Bt isolates with enhanced ability to replicate in the field, and Bt isolates with both Cry proteins and *Photorhabdus*-like toxins can be used for management of insects that develop resistance to Cry proteins alone.

Blackburn, M.B., Martin, P.A.W., Kuhar, D., Farrar, R.R., and Gundersen-Rindal, D.E. 2011. The occurrence of Photorhabdus-like toxin complexes in *Bacillus thuringiensis*. PLoS One 6:e18122. doi:10.1371/journal.pone.0018122.

Martin, P.A.W., Farrar, R.R., and Blackburn, M.B. 2009. Survival of diverse *Bacillus thuringiensis* strains in gypsy moth (Lepidoptera: Lymantriidae) is correlated with urease production. Biological Control 51:147–151.

Martin, P.A.W., Gundersen-Rindal, D.E., and Blackburn, M.B. 2009. Distribution of phenotypes among *Bacillus thuringiensis* strains. Systematic and Applied Microbiology 33:204-208.

Potential biopesticides against the coffee berry borer. The coffee berry borer (*Hypothenemus hampei*) is a curculionid beetle that is considered to be the worst insect pest of coffee. Originating in Africa, this pest has spread throughout coffee-growing regions of the world, most recently to Hawaii in 2010. ARS scientists in Beltsville, Maryland, sampled coffee from Mexico, Puerto Rico, Hawaii, and Colombia for endophytic fungi that would kill the coffee berry borer. They cultured fungi from the samples, identified fungi using molecular methods, and performed bioassays. As a result, the scientists found 16 species of insecticidal fungi in five genera (*Beauveria, Cladosporium, Acremonium, Conostachys,* and *Isaria*). These fungi are considered useful in the treatment of the coffee berry borer, especially since the fungi are pre-adapted to growing within the tissues of the coffee plant. Recent integrated pest management strategies against the borer have included the use of entomopathogenic fungi.

Vega, F.E., Posada, F., Aime, M.C., Pava-Ripoll, M., Infante, F., and Rehner, S.A. 2008. Entomopathogenic fungal endophytes. Biological Control 46:72-82.

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COMPONENT II: Protection of Agricultural and Horticultural Crops

In this Component, NP 304 scientists focused on protecting food and fiber crops from native and invasive insect, mite, and weed pests. There was an emphasis on developing cultural and biological approaches to pest control and improving traditional chemical-based tactics, with the long-term goal of producing sustainable, environmentally friendly practices. The scientists, often in collaboration with university and industry scientists, investigated broad problem areas, and the research outputs are used by growers; extension specialists; Federal, State, university, and industry researchers; and Federal regulators.

Over the past 5 years, the scientists in Component 2 made important discoveries, including a new insect-control bacterium and potent new aphicides based on novel modes of action. ARS scientists also developed major pest control technologies: mating disruption technology for navel orangeworm that saves almond and other tree nut growers $18 per acre; integrated pest management of the sweetpotato whitefly that has contributed to a 90-percent reduction in insecticide use; new fruit coloration guidelines that have eliminated one to two sprays, saving fruit growers as much as $61 per acre; and area-wide control program for tephritid fruit flies in Hawaii that has reduced the use of organophosphate insecticides by as much as 90 percent.

The NP 304 Action Plan included two Problem Statements that were expected to guide the 5-year research plan and the development of the anticipated products in this Component. The Problem Statements and the ARS research accomplishments that address each of them are presented below.

PROBLEM STATEMENT IIA: *Biology and Ecology of Pests and Natural Enemies*

Research on this Problem Statement focused on improving our understanding of the biology, ecology, and genetics of weeds, insects, and mites that threaten production of food and fiber crops. Projects during this 5-year period ranged from molecular studies that discovered new ways to control insects to landscape-level studies that improve pest control strategies. Notable accomplishments include the identification of genetic factors related to weedy characteristics, discovery of a plant-derived natural herbicide, discovery of a new bacterium that kills many key pest insects, and development of potent aphicides based on a novel mode of action.

Weeds

Genomics approaches help identify potential targets for manipulation of plant growth and development in perennial weeds. U.S. law requires land managers to control noxious perennial weeds such as Canada thistle and leafy spurge that spread and persist through vegetative reproduction from hundreds of underground adventitious buds on their extensive root systems. As dormancy in these buds helps the weed circumvent conventional control measures, ARS scientists in Fargo, North Dakota, and university and institute partners in the United States and Canada and at the International Institute of Tropical Agriculture in Ibadan, Nigeria, developed a suite of genomics tools to determine how environmental factors such as photoperiod,

temperature, and growth regulators impact molecular networks involved in regulating seasonal dormancy and flowering in these buds. This research highlighted molecular networks associated with transcription factors, circadian clock regulation, photo-morphogenesis, carbohydrate metabolism, and various hormones, which appear to impact cross-talk between dormancy and flowering pathways. In the case of Canada thistle, maintenance of para-dormancy in adventitious root buds highlighted the role of genes involved in interconnected plant hormone signaling networks. Elucidating the role of plant growth regulators on vegetative development was used to refine working models for dormancy regulation that will assist in the development of more effective, next-generation weed management strategies. These genomic tools have been applied by international scientists working to enhance the productivity of cassava, a staple food crop in developing countries.

Doğramaci, M., Foley, M.E., Chao, W.S., Christoffers, M.J., and Anderson, J.V. 2013. Induction of endodormancy in crown buds of leafy spurge (*Euphorbia esula* L.) implicates a role for ethylene and cross-talk between photoperiod and temperature. Plant Molecular Biology 81(6):577-593.

Anderson, J.V., Doğramaci, M., Horvath, D.P., Foley, M.E., Chao, W.S., Suttle, J.C., Thimmapuram, J., Hernandez, A.G., Ali, S., and Mikel, M.A. 2012. Auxin and ABA act as central regulators of developmental networks associated with paradormancy in Canada thistle (*Cirsium arvense*). Functional and Integrative Genomics 12(3):515-531.

Horvath, D.P., Sung, S., Kim, D., Chao, W.S., and Anderson, J.V. 2010. Characterization, expression and function of dormancy-associated Mads-box genes from leafy spurge. Plant Molecular Biology 73:169–179.

Horvath, D.P., Chao, W.S., Suttle, J.C., Thimmapuram, J., and Anderson, J.V. 2008. Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (*Euphorbia esula* L.). Biomed Central (BMC) Genomics 9:536.

Gene regulates seed dormancy and red grain color in weedy rice. To develop new weed management strategies, genes that regulate weedy characteristics such as seed dormancy need to be identified. Dormancy prevents germination, thus allowing weeds to elude control measures and persist year after year. Knowing that seed dormancy and red grain color are often coincident in cereal grains, ARS scientists in Fargo, North Dakota, in collaboration with university partners in South Dakota, China, and Japan, developed weedy rice as a genetic system to identify genes that regulate seed dormancy. The team discovered that a major gene that controls embryo dormancy explains about half of the variation in germination of weedy rice. They also discovered that a gene called Rc, which encodes a DNA transcription factor and codes for red grain color, regulates seed dormancy and germination by inducing the expression of genes for the biosynthesis of a dormancy inducing hormone called abscisic acid. This knowledge reveals new target sites to enhance and manipulate seed dormancy and germination, improve weed control strategies, and provide the means to reduce the incidence of pre-harvest sprouting in cereal grain crops.

Gu, X.Y., Foley, M.E., Horvath, D.P., Anderson, J.V., Feng, J., Zhang, L., Mowry, C.R., Ye, H., Suttle, J.C., Kadowaki, K.I., and Chen, Z. 2011. Association between seed dormancy and pericarp color is controlled by a pleiotropic gene that regulates abscisic acid and flavonoid synthesis in weedy red rice. Genetics 189:1515-1524.

Ye, H., Foley, M.E., and Gu X.Y. 2010. New seed dormancy loci detected from weedy rice-derived advanced populations with major QTL alleles removed from the background. Plant Science 179:612-619.

Gu, X.Y., Turnipseed, E.B., and Foley, M.E. 2008. The qSD12 locus controls offspring tissue-imposed seed dormancy in rice. Genetics 179:2263-2273.

Mechanisms of weed-induced yield losses identified. Weeds use a variety of competitive strategies that reduce crop yields. ARS researchers in Fargo, North Dakota, and Stoneville, Mississippi, uncovered novel genetic mechanisms that do not seem to result from competition for resources as previously assumed, but rather from weeds altering expression of genes involved in growth and development in the crop that eventually leads to lower yield. In collaboration with university partners in South Dakota, ARS scientists found that when velvetleaf (*Abutilon theophrasti*) or canola (*Brassica rapa*) grew in proximity with corn, the expression of photosynthesis genes such as Photosystem II stability/assembly factor HCF136 were reduced in corn. Additionally, researchers found that the drought-responsive gene RD22 is up-regulated in corn in the presence of weeds. These discoveries are paving the way for further research to make crops "blind" to weeds by interfering with the way nearby weeds cause changes in growth and photosynthesis in crops, ultimately protecting crops from yield losses. This work has resulted in two papers that have been recognized as "paper of the year" by the Weed Science Society of America in 2007 and 2011.

Moriles, J., Hansen, S., Horvath, D.P., Reicks, G., and Clay, D.E., Clay, S.A. 2011. Microarray and growth analyses identify differences and similarities of early corn response to weeds, shade, and nitrogen stress. Weed Science 60:158–166.

Clay, D.E., Clay, S.A., Horvath, D.P., Pullis, J., Carlson, C.G., Hansen, S., and Reicks, G. 2009. Corn (*Zea mays*) response to competition: growth alteration vs. yield limiting factors. Agronomy Journal 101:1522-1529.

Horvath, D.P. and Clay, S. 2007. Heterologous hybridization of cotton (*Gossypium hirsutum*) microarrays with velvetleaf (*Abutilon theophrasti*) reveals physiological responses due to corn competition. Weed Science 55:546–557.

Transgenic modification of synthesis of the allelochemical sorgoleone in sorghum. Plant-derived natural products represent attractive alternatives to synthetic herbicides, and the ability to engineer or manipulate their biosynthesis in crop plants could significantly reduce reliance on synthetic herbicides input in agricultural systems. Basic work on the physiology of root hairs by ARS scientists in Oxford, Mississippi, led to the discovery of a potent herbicidal allelochemical called sorgoleone, a compound found only in sorghum plants. Constructing a DNA library from sorghum root hair cells, these scientists identified the genetic sequences responsible for production and genetic regulation of sorgoleone, followed by successful insertion and expression of several of the genes in rice and mustard plants, which demonstrates that unrelated plants could be genetically engineered with this potential natural herbicide. In addition, researchers characterized all of the enzymes required for the biosynthesis of sorgoleone *in vivo*. The work also resulted in the generation of the first sorgoleone-deficient sorghum plants, produced via RNAi-mediated technology. In addition to providing a toolbox for manipulating the production of sorgoleone *in planta*, the identified genes could be used for manipulating the production of related compounds, such as phytoanticipins (anti-microbial compounds produced constitutively in plants) and potent allelochemicals that combat weeds. Furthermore, the scientists discovered

a novel desaturase involved in an early step of sorgoleone biosynthesis that is potentially useful for the engineering of oilseed crops to produce nutritionally beneficial and/or commercially valuable fatty acids.

Pan, Z., Rimando, A.M., and Baerson S.R. 2013. Genes encoding fatty acid desaturases from *Sorghum bicolor*. U.S. Patent and Trademark Office, Patent no. 8,383,890. February 2013.

Baerson, S.R., Rimando, A.M., Dayan, F.E., Pan, Z., and Polashock, J.J. 2010. O-methyltransferase gene from sorghum. Cloning, expression, transformation and characterization. U.S. Patent and Trademark Office, Patent no. 7,732,666, June 2010.

Cook, D., Rimando, A.M., Clemente, T.E., Schröder, J., Dayan, F.E., Nanayakkara, N.P., Pan, Z., Noonan, B.P., Fishbein, M., Abe, I., Duke, S.O., and Baerson, S.R. 2010. Alkylresorcinol synthases expressed in *Sorghum bicolor* root hairs play an essential role in the biosynthesis of the allelopathic benzoquinone sorgoleone. Plant Cell 22:867-87.

Baerson, S.R., Dayan, F.E., Rimando, A.M., Nanayakkara, N.P. D., Liu, C., Schroeder, J., Fishbein, M., Pan, Z., Kagan, I.A., Pratt, L.H., and Duke, S.O. 2008. A functional genomics investigation of allelochemical biosynthesis in Sorghum bicolor root hairs. Journal of Biological Chemistry 283:3231-3247.

Insights into molecular interactions of regulatory protein and phytotoxins. A major challenge in the use of chemicals to control weeds is the limited number of available modes-of-action of existing herbicides. However, many plant toxins produced by bacteria, particularly *Pseudomonas syringae* strains, have herbicidal modes-of-action unlike those of most conventional herbicides, although production levels of these natural herbicides are currently insufficient to warrant commercialization. Using molecular genetics approaches, ARS researchers in Beltsville, Maryland, in collaboration with molecular biologists at the University of Nottingham, United Kingdom, demonstrated that overproduction of RsmA, a regulatory protein produced by the common bacteria *P. aeruginosa*, turns off phytotoxin production in three unrelated strains of *P. syringae*. Use of this newly discovered mode-of-action in multiple strains of *P. syringae* bacteria will help researchers improve phytotoxin production to commercially acceptable levels and/or improve the bio-herbicidal activity of *P. syringae* strains that may be useful in the biological control of weeds.

Kong, H.S., Roberts, D.P., Patterson, C.D., Kuehne, S.A., Heeb, S., Lakshman, D.K., and Lydon, J. 2012. Effect of over-expression of RsmA from *Pseudomonas aeruginosa* on virulence of select phytotoxin-producing strains of *Pseudomonas syringae*. Phytopathology 102:575-587.

Technology solving herbicide sensitivity in sweet corn. Herbicide sensitivity has been considered the most serious pest management issue in sweet corn production and an intractable problem since the early 1990s. Breakthroughs in elucidating the molecular and biochemical mechanisms of herbicide sensitivity in sweet corn have led to declining injury and yield losses. ARS scientists in Urbana, Illinois, in collaboration with University of Illinois scientists, discovered that a single recessive gene—or a group of very closely linked genes—causes sweet corn to have cross-sensitivity to 11 herbicides, with five different modes of action in this group. The research team identified the chromosomal location of the gene(s), a cytochrome (CYP) named P450 at locus 5S, and showed how interactions among weather, herbicide formulation, and the CYP genotypic class influenced crop response to several P450-metabolized herbicides.

Furthermore, all 12 U.S. commercial sweet corn breeding programs were shown to have lines that carry herbicide-sensitive CYP alleles. Several commercial companies are now developing new corn lines with herbicide tolerance by modifying CYP alleles. The herbicide manufacturing industry has adopted this technology to determine risks that their products pose to the $1 billion sweet corn industry. Preliminary reports indicate that injury and yield loss to U.S. sweet corn from herbicides is being reduced nationally.

Williams II, M.M. and Pataky, J.K.. 2010. Factors affecting differential sweet corn sensitivity to HPPD-inhibiting herbicides. Weed Science 58:289-294.

Pataky, J.K., Williams, M.M. II, Riechers, D.E., and Meyer, M.D. 2009. A common genetic basis for cross-sensitivity to mesotrione and nicosulfuron in sweet corn hybrids and inbreds grown throughout North America. Journal of the American Society for Horticultural Science 134:252-260.

Nordby, J.N., Williams II, M.M., Pataky, J.K., Riechers, D.E., and Lutz, J.D. 2008. A common genetic basis in sweet corn inbred Cr1 for cross-sensitivity to multiple cytochrome P450-metabolized herbicides. Weed Science 56:376-382.

Pataky, J.K., Meyer, M.D., Bollman, J.D., Boerboom, C.M., and Williams II, M.M. 2008. Genetic basis for varied levels of injury to sweet corn hybrids from three cytochrome P-450 metabolized herbicides. Journal of the American Society for Horticultural Science 133:438-447.

EPSPS gene amplification a key resistance mechanism. Palmer amaranth (*Amaranthus palmeri*), a serious weed in row crops such as cotton and soybean, has developed resistance to glyphosate and has spread rapidly to 15 states, requiring farmers to seek alternative control measures that will increase production costs and potentially reduce crop yields. ARS scientists in Stoneville, Mississippi, determined the nature of the amplification event that resulted in high levels of glyphosate resistance in the weed. The researchers discovered that the amplicon (gene sequence) for resistance contains genetic elements that promote or permit uncontrolled synthesis of the target site protein of glyphosate, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Although regulation of these genetic elements appears to have been compromised, the intricacies of this process are not yet characterized. Recently, this same mechanism was identified in glyphosate-resistant Italian ryegrass, a weed of equal importance. This herbicide resistance mechanism, coupled with the aggressiveness of Palmer amaranth, threatens the utility of glyphosate-resistant cropping systems. This research provides insight into the molecular mechanism of herbicide resistance, critical information that can be applied to improved control of this economically important weed.

Gaines, T.A., Wright, A.A., Molin, W.T., Lorentz, L., Riggins, C. W., Tranel, P.J., Beffa, R., Westra, P., and Powles, S.B. 2013. Identification of genetic elements associated with EPSPS gene amplification. PLoS ONE 8(6):e65819. DOI:10.1371/journal.pone.0065819.

First report of a target-site mutation in a glyphosate-resistant dicot weed. Tall waterhemp and Palmer amaranth have been reported to hybridize, thereby creating weeds with increased resistance to glyphosate. Growers or land managers could be confronted with populations of tall waterhemp, Palmer amaranth, or a mixture of these weeds along with hybrids that may contain genes encoding multiple mechanisms of resistance, thereby greatly increasing input costs for weed control and/or decreasing crop yields. ARS scientists in Stoneville, Mississippi, have

determined that the mechanism of resistance to glyphosate in tall waterhemp is due to a combination of a target-site (EPSPS) mutation and a non-target site (non-EPSPS)-based mechanism. This is the first report of an altered target site mutation in EPSPS in a dicot weed species that has evolved resistance to glyphosate. Also, glyphosate-resistant waterhemp plants absorbed and translocated significantly less glyphosate compared to the glyphosate-susceptible population, which is considered a non-target site resistance mechanism. These findings provide evidence of the pitfalls of relying on a sole weed management technique (glyphosate-resistant crops) and highlights the need for diversification of weed management portfolios. As an alternative, post-emergence applications of paraquat, glufosinate, and fomesafen and pre-emergence treatment with fomesafen, *s*-metolachlor, and pendimethalin effectively controlled 100 percent of glyphosate-resistant Palmer amaranth plants in Mississippi. These herbicides, representing different modes-of-action, could be used as an alternative for managing glyphosate-resistant Palmer amaranth, particularly if accompanied by responsible stewardship programs.

Nandula, V.K., Ray, J.D., Ribeiro, D.N., Pan, Z., and Reddy, K.N. 2013. Glyphosate resistance in tall waterhemp (*Amaranthus tuberculatus*) from Mississippi is due to both altered target site and non-target site. Weed Science 61(3):374-383.

Nandula, V.K., Reddy, K.N., Koger, C.H., Poston, D.H., Rimando, A.M., Duke, S.O., Bond, J.A., and Ribeiro, D. N. 2012. Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. Weed Science 60(2):179-188.

Restoration using annual cover crops does not inhibit establishment of perennials. Annual grasses such as oats and millet are routinely planted in soils that have been severely disturbed to provide a ready source of forage for cattle and serve as cover crops to help prevent soil erosion. However, many land managers have concerns that these annual grasses could prevent successful establishment of simultaneously seeded, long-lived perennial grasses by competing for resources such as light, water, and soil nutrients. In both greenhouse and field experiments on a farm and ranchland near Sidney, Montana, that were severely disturbed by a large pipeline project, ARS researchers in Sidney demonstrated that annual grass seeds did not prevent perennial grasses from germinating or emerging, and annual seedlings did not slow the growth of perennial seedlings, as would be expected if the two groups of plants were competing for resources. Severe competition from annuals was only indicated in greenhouse tests in well-watered farm soils (both fertilized and unfertilized). The researchers also showed that, under most conditions, annual cover crops do not interfere with perennial grass establishment and growth; when soils have high levels of sodium and sulfur (which can result from pipeline projects), cover crops may even facilitate perennial establishment. In addition, annual grasses ameliorated the effects of water stress under drought conditions by improving water infiltration to soils and providing shade to soils, which reduced water loss from evaporation. This research provides the first sound scientific evidence of the benefits of using annuals in revegetating large plots of land disturbed by large pipeline projects. Future research will determine whether seeding with annual grasses can mitigate the effects of grazing pressure on the establishment of perennial grasses for the sustainable use of rangelands for grazing.

Espeland, E.K. and Perkins, L.B. 2013. Annual cover crops do not compete with perennial grasses on a disturbed restoration soil in the Northern Great Plains USA. Ecological Restoration 31(1):69-78.

Insects

Whitefly-host plant-virus relationships in sweet potato. In an early 1990s outbreak , whitefly-transmitted viruses in vegetable crops cost the United States $500 million in damage each year. An understanding of relationships among whiteflies, whitefly transmitted viruses, and their host plants is needed to strategically protect sweet potato plants from insect vectors and their associated viruses. ARS scientists in Charleston, South Carolina, conducted multidisciplinary research to determine interrelationships and importance of sweet potato leaf curl virus, which is transmitted by the sweet potato whitefly, *Bemisia tabaci*, to sweet potato. Research quantified whitefly virus transmission efficiency, acquisition and retention of the virus, and host range. Host range of the sweet potato leaf curl virus was extended to an additional 29 species (weeds in the same genus, *Ipomoea*), and 55 new host plants for *B. tabaci* were identified. The research further revealed that plants of many sweet potato varieties and breeding lines do not display sweet potato leaf curl virus infection symptoms. Symptoms of infected plants ranged from asymptomatic to dramatic leaf curling, but all evaluated germplasm became infected with the virus in the field. Yield among sweet potato germplasm was found to be reduced by 10-80 percent in response to the virus. Insecticide sprays were largely ineffective in reducing whiteflies to a level where transmission of the sweet potato leaf curl virus was reduced. A related study concerning the tomato yellow leaf curl virus in tomato involved Federal and State researchers in South Carolina, Georgia, and Florida. This work provides basic information on the virus and its vector and their interactions with crops, information needed to develop future management strategies such as resistant crop lines. This research also led to a study on the effect of global warming on whiteflies supported by the Bill and Melinda Gates Foundation.

Ling, K., Harrison Jr., H.F., Simmons, A.M., Zhang, S., and Jackson, D.M. 2011. Experimental host range and natural reservoir of Sweet potato leaf curl virus in the United States. Crop Protection 30:1055-1062.

Ling, K., Jackson, D.M., Harrison Jr., H.F., Simmons, A.M., and Pesic-Valesbroeck, Z. 2010. Field evaluation of yield effects on the U.S.A. heirloom sweet potato cultivars infected by Sweet potato leaf curl virus. Crop Protection 29:757-765.

Simmons, A.M., Ling, K., Harrison Jr., H.F., and Jackson, D.M. 2009. Sweet potato leaf curl virus: Efficiency of acquisition, retention and transmission by *Bemisia tabaci* (Hemiptera: Aleyrodidae). Crop Protection 28:1007-1011.

Simmons, A.M., Harrison Jr., H.F., and Ling, K. 2008. Forty-nine new host plant species for *Bemisia tabaci* (Hemiptera: Aleyrodidae). Entomological Science (Japan) 11:385-390.

Improved methods for molecular analysis of arthropod predator-prey interactions. Molecular methods have revolutionized the collection of gut-content data and revealed important insights into the role of predators as natural enemies of insect pests. However, variability in the sizes of prey individuals and in the time between consumption of a prey item and collection of the predator that consumed it make translation of raw gut-content assay data into ecologically meaningful rates of predation problematic. In collaboration with scientists at Oklahoma State University and ARS laboratories in Brookings, South Dakota, and Columbia, Missouri, ARS scientists in Beltsville, Maryland, developed the “detectability half-life” of a standard quantity of prey DNA in the gut, enabling correction of raw data for biases due to differences in detectability of a given prey species in the guts of different predators. Half-lives were determined for the

entire suite of major predators attacking Colorado potato beetle, which enabled the scientists to rank the predators in importance. These adjusted rankings showed that the spined soldier bug, a generalist, was the most important Colorado potato beetle predator on a per-capita basis, ahead of two specialists, the two-spotted stink bug and the *Lebia* ground beetle, while the most abundant predator, the spotted pink ladybeetle, had the smallest impact. This, in conjunction with field density data, clarified the role of predators in suppressing this devastating pest, providing valuable information to scientists and IPM managers on how best to manage the natural enemy complex. Concurrent with this work, the ARS scientists determined the influence of prey number, time since consumption, consumption of subsequent prey, and method of preservation on the quantity of DNA in a predator's gut. They also developed a simple protocol for removing external DNA contamination from field-collected predators. The detectability half-life is now widely employed by molecular ecologists in Australia, Europe, and the United States, and the DNA decontamination protocol has been adopted by scientists at other government research agencies and in Australia.

Greenstone, M.H., Weber, D.C., Coudron, T.C., Payton, M.E., and Hu, J.S. 2012. Removing external DNA contamination from predators destined for molecular gut-content analysis. Molecular Ecology Resources 12:464-469.

Greenstone, M.H., Szendrei, Z., Rowley, D.L., Payton, M.E., and Weber, D.C. 2010. Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle. Entomologia Experimentalis et Applicata 136:97-107.

Greenstone, M.H., Weber, D.C., Coudron, T.C., and Payton, M.E. 2010. Unnecessary roughness? Testing the hypothesis that predators destined for molecular gut-content analysis must be hand-collected to avoid cross-contamination. Molecular Ecology Resources 11:286-293.

Szendrei, Z., Greenstone, M.H., Payton, M.E., and Weber, D.C. 2010. Molecular gut-content analysis of a predator assemblage reveals the effect of habitat manipulation on biological control in the field. Basic and Applied Ecology 11:153-161.

Weber, D.C. and Lundgren, J.G. 2009. Detection of predation using qPCR: Effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. Journal of Insect Science 9:41 (12pp.), available online: insectscience.org/9.41.

Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E., and Hawthorne, D.J. 2007. Feeding mode and prey detectability half-lives in molecular gut-content analysis: An example with two predators of the Colorado potato beetle. Bulletin of Entomological Research 97:201-209.

Identification of functional aquaporins in guts of sucking insect pests. Complexes of sucking insects (whiteflies, plant bugs, and stink bugs) are inherently difficult to manage in cotton and other crops because of their mobility, utilization of numerous crop and non-crop host plants, and propensity to develop resistance to conventional insecticides. Ecologically based management tactics such as manipulation of cropping patterns are useful, but they are difficult to implement on a community wide scale and would be more effective in concert with other, non-insecticidal tactics. ARS scientists in Maricopa, Arizona, and their collaborators identified and characterized a novel aquaporin water transport protein from the gut of the sweet potato whitefly. The

aquaporin is localized in a specialized gut structure where it plays a critical role in regulating water balance and excretion. The work, which was to first to demonstrate a functional aquaporin in the whitefly gut, identified a logical target for disruption by molecular-based control tactics such as RNA interference. Results contribute to a more complete knowledge of insect digestive biochemistry and physiology and will accelerate discovery of similar molecular targets in whitefly and other sucking pests. Two cooperative agreements with scientists in Australia and at the University of Arizona have been established to identify and develop inhibitors of aquaporins for use in pest management.

Mathew, L.G., Campbell, E.M., Yool, A.J., and Fabrick, J.A. 2011. Identification and characterization of a functional aquaporin water channel protein from alimentary tract of whitefly, *Bemisia tabaci*. Insect Biochemistry and Molecular Biology 41(3):178-190.

New markers, genomic insights, host prediction information, and landscape-level dispersal and population dynamics models for Western tarnished plant bug. With adoption of transgenic field crops and consequent reduction in the use of pesticides on these crops, piercing sucking insects such as the Western tarnished plant bug, L*ygus hesperus,* and related species not affected by the toxins in the transgenic crops, have emerged as major pests of field, fruit, and vegetable crops in the United States. Current controls for plant bugs rely heavily on conventional insecticides, but continued effectiveness of these materials is threatened by the development of insecticide resistance. Development of alternative management tactics will require detailed understanding of plant bug ecology, physiology, and chemical communication. Means are needed to follow the movement, host preference, and population dynamics of this pest, as well as genomic knowledge that might reveal Western tarnished plant bug vulnerabilities. ARS scientists in Maricopa, Arizona, have discovered key chemical signals that act in a complex network to regulate mating and reproduction in the Western tarnished plant bug. This regulatory network is unlike those so far reported for other insect groups. Males of this species can render a female unattractive to other males by transferring two compounds to a female when they mate. Although this "anti-aphrodisiac" effect has been observed in other insects, only in the Western tarnished plant bug has it been found that females will take one of these compounds and chemically convert it into a substance that, while not itself an attractant, is able to counteract the negative effect of the other two compounds (an "anti-anti-aphrodisiac"). The added complexity of this communication system provides additional opportunities for disrupting a key part of the reproductive processes of these insects.

Brent, C.S., Klok, C.J., and Naranjo, S.A. 2013. Effect of diapause status and gender on activity, metabolism and starvation resistance in the plant bug *Lygus hesperus* Knight. Entomologia Experimentalis et Applicata 148:152–160.

Brent, C.S. and Byers, J.A. 2011. Female attractiveness modulated by a male-derived antiaphrodisiac pheromone in a plant bug. Animal Behavior 82(5):937-943.

Brent, C.S., Fasnacht, M.P., and Judd, T.M. 2011. Post-mating enhancement of fecundity in female *Lygus hesperus*. Physiological Entomology 36(2):141-148.

Brent, C.S. 2010. Reproduction of the Western Tarnished Plant Bug, *Lygus hesperus*, in relation to age, gonadal activity and mating status. Journal of Insect Physiology 56(1):28-34.

To better track the Western tarnished plant bug, ARS scientists in Maricopa, Arizona, identified and evaluated the use of widely available protein markers, including egg albumin in egg white, casein in cow's milk, and soy trypsin inhibitor in soy milk, as substitutes for vertebrate IgG proteins. The alternative markers are less expensive and sometimes more effective than traditional IgG proteins. The inexpensive protein markers (second generation) cost $0.25 per liter to apply, compared to $500 per liter for the first-generation protein markers. Reduced cost of alternative markers facilitates large-scale studies that were previously prohibitively expensive and increases availability of protein-marking methodology to the research community for this and other pests. Development of low-cost markers has prompted collaborations and consultations on objectives ranging from plant bug movement in organically grown strawberries to species composition and dispersal of arthropods in potential biofuels crops in the southern United States. Trap cropping with alfalfa has been shown to be an effective management approach for control of *Lygus* spp. in organic strawberries in California, where more than 25 percent of the acreage in some production regions now deploy the strategy. ARS research on insect dispersal is unraveling the pest behavioral elements important to the trap crop approach.

Swezey, S.L., Nieto, D.J., Hagler, J.R., Pickett, C.H., Bryer, J.A., and Machtley, S.A. 2013. Dispersion, distribution, and movement of *Lygus* spp. (Hemiptera: Miridae) in trap-cropped organic strawberries. Environmental Entomology 42(4):770-778.

Williams III, L., Hagler, J.R., and Tonkel, K.C. 2013. Retention of immunlabels by *Diorhabda carinulata*, a biological control agent of saltcedar. Entomologia Experimentalis et Applicata 141:154-162.

Kelly, J.L. Hagler, J.R., and Kaplan, I. 2012. Employing immunomarkers to track dispersal and trophic relationships of a piercing-sucking predator, *Podisus maculiventris* (Hemiptera: Pentatomidae). Environmental Entomology 41(6):1527-1533.

Irvin, N.A., Hagler, J.R., and Hoddle, M.S. 2012. Laboratory investigation of triple marking the parasitoid, *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) with a fluorescent dye and two animal proteins. Entomologia Experimentalis et Applicata 143(1):1-23.

Krugner, R., Hagler, J.R., Groves, R.L., Sisterson, M.S., Morse, J.G., and Johnson, M.W. 2012. Plant water stress effects on the net dispersal rate of the insect vector *Homalodisca vitripennis* (Hemiptera: Cicadellidae) and movement of its egg parasitoid, *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae). Environmental Entomology 41(6):1279-1289.

Baker, P.B., Hagler, J.R., Marchosky, R., Machtley, S.A., Brown, J.M., Riehle, M.A., and Bellamy, D.E. 2011. Utilizing rabbit immunoglobulin G protein for mark-capture studies on the desert subterranean termite, *Heterotermes aureus* (Snyder). Insectes Sociaux 57:147-155.

Hagler, J.R. 2011. An immunological approach to quantify consumption of protein-tagged *Lygus hesperus* by the entire cotton predator assemblage. Biological Control 58(3):337-345.

Hagler, J.R., Mueller, S., Teuber, L.R., Van Deynze, A., and Martin. J. 2011. A method for distinctly marking honey bees, *Apis mellifera*, originating from multiple apiary locations. Journal of Insect Science 11:143.

Hagler, J.R., Mueller, S., Teuber, L.R., Machtley, S.A., and Van Deynze, A. 2011. Foraging range of honey bees, *Apis mellifera*, in alfalfa seed production fields. Journal of Insect Science 11:144.

Hagler, J.R. and Jones, V.P. 2010. An approach to mark arthropods for mark capture type research. Entomologia Experimentalis et Applicata 135(2):177-192.

ARS scientists in Maricopa and their collaborators conducted field studies to define seasonal and spatial patterns of the Western tarnished plant bug populations within complex agroecosystems and, using this information, constructed models to predict risks from plant bugs in specific cotton fields based on proximity and composition of surrounding crops that act as sources and sinks of dispersing insects. A user-friendly computer simulation was constructed as a tool for educating growers and industry representatives regarding the consequences landscape-level cropping patterns. Results allow producers to evaluate and adopt cropping patterns that reduce crop injury from plant bugs on a community wide scale. The resulting information is currently being transferred to stakeholders through Cooperative Extension training.

Carrière, Y., Goodell, P.B., Ellers-Kirk, C., Larocque, G., Dutilleul, P., Naranjo, S.E., and Ellsworth, P.C. 2012. Effects of local and landscape factors on population dynamics of a cotton pest. PLoS ONE 7(6):e39862.

Sivakoff, F.S., Rosenheim, J.A., and Hagler, J.R. 2012. Relative dispersal ability of a key agricultural pest and its predators in an annual agroecosystem. Biological Control 63(3):296-303.

Previous USDA efforts to manage the Western tarnished plant bug on an area-wide basis in the Mississippi Delta demonstrated that populations in cotton could be affected and insecticide use reduced by 50 percent or more when efforts to eliminate early-season wild hosts were coordinated around cotton production. This work was done before recent expansion of corn production and wide-scale planting of indeterminate, early production system soybean varieties in the southern United States. Using stable carbon isotope methods, ARS scientists in Stoneville, Mississippi, showed that a majority of insects colonizing cotton early in the growing season originate from immatures feeding on C4 hosts. Corn is the predominant C4 host in the Mississippi Delta, but a number of pigweed species (*Amaranthus* spp.) are preferred hosts, along with other C4 plants. Pigweed is becoming increasingly resistant to glyphosate, and abundance of the weed is increasing on field borders and within all production fields in the Delta. This potentially impacts distribution of the Western tarnished plant bug across the landscape. Combining stable nitrogen isotope analyses with stable carbon analyses provided a method to differentiate bugs originating from corn and pigweed. This is key information for identification of the critical links in population growth of the Western tarnished plant bug that can be attacked to lessen densities colonizing cotton and eliminate the current excessive use of insecticides based on reactionary control of this polyphagous pest. The scientists have since confirmed that the Western tarnished plant bug feeds and reproduces

on corn and that populations can be extremely high on early production system soybean. This impacts utility of previous area-wide management strategies because the pest is more spatially distributed across the agricultural landscape.

Jackson, R.E., Snodgrass, G.L., Allen, K.C., Perera, O.P., and Price, L.D. 2012. Analysis of carbon and nitrogen isotopes for natal host determination of tarnished plant bug (Hemiptera: Miridae) adults. Southwestern Entomologist 37:123-132.

Abel, C.A., Snodgrass, G.L., Jackson, R.E., and Allen, K.C. 2010. Oviposition and development of the tarnished plant bug (Hemiptera: miridae) on field maize. Environmental Entomology 39(4):1085-1091.

Snodgrass, G.L., Jackson, R.E., Abel, C.A., and Perera, O.P. 2010. Utilization of early soybeans for food and reproduction by the tarnished plant bug (Heteroptera: Miridae) in the Delta of Mississippi. Environmental Entomology 39(4):111-121.

Although the Western tarnished plant bug attacks a wide variety of crops in the desert Southwest, including potential new biofuel crops such as camelina, vernonia, and lesquerella, the potential impacts of plant bugs on these crops are unknown. ARS scientists in Maricopa, Arizona, and their collaborators observed plant bug densities in these crops that were similar in magnitude to populations on other preferred hosts in the region. Of the three alternative crops, vernonia was most preferred for feeding and egg-laying, whereas camelina was least preferred. Field studies indicated that plant bugs fed on lesquerella blooms and seed pods, but when the crop received optimal levels of irrigation and fertilization and weeds were effectively controlled, plant bugs did not decrease seed yield or quality. The results highlight the importance of proper agronomic management of the new industrial crops and suggest that commercial-level production may influence levels and distributions of plant bug populations in the larger agricultural landscape. Findings have been provided to key pilot producers and will enhance their efforts to commercialize these alternative crops, with guayule acreage expected to expand up to 200,000 acres from the few thousand acres in Arizona today.

Naranjo, S.E., and Stefanek, M.A. 2012. Feeding behavior of a potential insect pest, *Lygus hesperus*, on four new industrial crops for the arid southwestern USA. Industrial Crops and Products 37(1):358-361.

Naranjo, S.E., Ellsworth, P.C., and Dierig, D.A. 2011. Impact of *Lygus* spp. (Hemiptera: Miridae) on damage, yield and quality of lesquerella (*Physaria fendleri*), a potential new oil-seed crop. Journal of Economic Entomology 104(5):1575-1583.

Blackmer, J.L. and Byers, J.A. 2009. *Lygus* spp. (Heteroptera: Miridae) host-plant interactions with *Lesquerella fendleri* (Brassicaceae), a new crop in the arid Southwest. Environmental Entomology 38(1):159-167.

ARS scientists in Maricopa and collaborators also constructed the first transcriptome for the Western tarnished plant bug. The scientists cloned specific genes linked to perception of chemical communication, mating regulation, and thermal stress response, which may be important to plant bug survival in the arid Southwest. The transcriptome represents the first step toward identification and exploration of molecular targets for disruption by control tactics and has received ongoing support from Cotton Inc**.**

Hull, J.J, Hoffmann, E.J., Perera, O.P., and Snodgrass, G.L. 2012. Identification of the western tarnished plant bug (*Lygus hesperus*) olfactory co-receptor Orco: Expression profile and confirmation of atypical membrane topology. Archives of Insect Biochemistry and Physiology 81(4):179-198.

Identification of pheromone receptors in Lepidoptera. To further enhance use and effectiveness of attractants in IPM, researchers seek to identify and develop other types of semiochemicals, such as pheromone analogs and disruptors, and kairomones. One approach is through the identification and characterization of protein receptors involved in olfaction (cumulatively referred to as pheromone receptors) and their genes. Most pheromone receptors have been identified from genomes or transcriptomes, an approach that has been costly and slow. ARS scientists in Wapato, Washington, developed a cost-effective, targeted technique to identify pheromone receptors expressed by codling moth, a major pest of apple. In collaboration with researchers at Montana State University and the University of California at Davis, the scientists demonstrated that the technique can be applied to easily and cheaply identify pheromone receptors in other Lepidopteran pests. To date, the researchers have used the technique to identify more than 75 pheromone receptors from 29 species of Lepidoptera. Results of this research are now being applied to unravel the molecular basis of moth odorant receptor-pheromone interactions, with the goal of facilitating the development of additional and more effective semiochemical-based pest control strategies.

Garczynski, S.F., Wanner, K.W., and Unruh, T.R. 2012. Identification and initial characterization of the 3' end of gene transcripts encoding putative members of the pheromone receptor subfamily in Lepidoptera. Insect Science 19:64-74.

Xu, P., Garczynski, S.F., Atungulu, E., Syed, Z., Choo, Y.-M., Vidal, D.M., Zitelli, C.H.L., and Leal, W.S. 2012. Moth sex pheromone receptors and deceitful parapheromones. PLoS ONE 7:e41653.

Wanner, K.W., Nichols, A.S., Allen, J.E., Bunger, P.L., Garczynski, S.F., Linn, C.E., Robertson, H.M., and Luetje, C.W. 2010. Sex pheromone receptor specificity in the European corn borer moth, *Ostrinia nubilalis*. PLoS One 5:e8685.

Influence of dietary trace metals on insect performance. Some metals, such as iron, copper, zinc, selenium, and manganese, are essential dietary nutrients for vertebrates and invertebrates. These trace metals are usually associated with various proteins, and when lacking in food, the metal-requiring proteins lose their functionality, to the detriment of the animals. The insect requirements for trace metals have been recognized for decades; however, specific physiological functions that depend on trace metals had not been previously identified. ARS scientists in Columbia, Missouri, in collaboration with scientists at the University of Missouri Research Reactor, discovered that insect immune functions depend on adequate levels of these dietary trace metals. This new information prompted commercial firms that formulate insect cell culture media to alter trace metal concentrations, leading to improved production of proteins for biomedical use in insect cell culture-based protein production systems.

Cheruiyot, D.J., Boyd, R.S., Coudron, T.A., and Cobine, P. 2013. Effect of biotransfer and bioaccumulation of herbivore dietary Co, Cu, Ni and Zn on growth and development of the insect predator *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Journal of Chemical Ecology 39:764-772.

Coudron T.A., Mitchell L.C., Sun R., Robertson J.D., Nha P., and Popham H.J.R. 2012. Dietary composition affects levels of trace elements in *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Biological Control 61:141-146.

Popham H.J.R., Sun R., Shelby K.S., and Robertson J.D. 2012. Iron levels change in larval *Heliothis virescens* tissues following baculovirus infection. Biological Trace Element Research 148:356-362.

Popham H.J.R., Sun R., Shelby K.S., and Robertson J.D. 2012. Changes in trace metals in hemolymph of baculovirus-infected noctuid larvae. Biological Trace Element Research 146:325-334.

Fall armyworm as a model system to study effect of climate change on long-distance migration. A major consequence of long-term climate change will be the geographical redistribution of agricultural pest insects due to altered migratory behaviors. ARS scientists in Gainesville, Florida, and College Station, Texas, with researchers at Pennsylvania State University, have establishd a national network for monitoring the fall armyworm—a major lepidopteran pest of corn and other crops in the Western Hemisphere—that now includes over 120 co-operators in 29 states and additional sites in Mexico and Canada. The researchers are using a novel methodology involving mitochondrial haplotype frequencies together with pheromone type and pheromone-based traps that led to the delineation of fall armyworm biotypes and migration pathways. Collection data are being incorporated into PestWatch (www.pestwatch.psu.edu), a Web-based interactive network that monitors annual movements of pests and plant diseases. With this information, new strategies are being designed to reduce the populations of migrating armyworms, such as the use of legume cover crops that will reduce plant host availability in critical overwintering locations. The use of cover crops will decrease pesticide use while fixing nitrogen and improving soil sustainability in areas adversely impacted by climate change. Success of the project has generated international interest, with additional collaborations established with researchers in Argentina, Brazil, Mexico, and the Caribbean.

Meagher, R.L. and Nagoshi, R.N. 2013. Attraction of fall armyworm (Lepidoptera: Noctuidae) to host strain females in Florida. Environmental Entomology 42(4):751-7.

Unbehend, M., Hänniger, S., Meagher R., Heckel, D., and Groot, A. 2013. Pheromonal divergence between two strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Journal of Chemical Ecology 39(3):264-276.

Nagoshi, R.N., Meagher, R.L., and Hay-Roe, M. 2012. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. Ecology and Evolution 2:1458-1467.

Nagoshi, R.N., Meagher, R.L., and Jenkins, D.A. 2010. Puerto Rico fall armyworm has only limited interactions with those from Brazil or Texas but could have substantial exchanges with Florida populations. Journal of Economic Entomology 103:360-367.

Nagoshi, R.N., Fleischer, S.J., and Meagher, R.L. 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: Implications for migration into the northeastern United States. Environmental Entomology 38:1546-1554.

Nagoshi, R.N., Armstrong, J.S., Silvie, P., and Meagher, R.L. 2008. Structure and distribution of a strain-biased tandem repeat element in fall armyworm (Lepidoptera: Noctuidae) populations in Florida, Texas, and Brazil. Annals Entomological Society of America 101:1112-1120.

Nagoshi, R.N., Meagher, R.L., Flanders, K., Gore, J., Jackson, R., Lopez, J., Armstrong, J.S., Buntin, G.D., Sansone, C., and Leonard, B.R. 2008. Using haplotypes to monitor the migration of fall armyworm (Lepidoptera:Noctuidae) corn-strain populations from Texas and Florida. Journal of Economic Entomology 101:742-749.

Chemical concentration learning in a parasitic wasp. Surgical castration of pigs has been long used to prevent taint in meat from male pigs, which is a large problem in the pig husbandry industry. Due to obvious animal welfare issues, the European Union now wants an alternative to castration, which creates a need for novel methods of boar taint detection. As boar taint is only a problem when taint chemicals exceed a well-defined threshold, detection methods should be concentration specific. Parasitic wasps, *Microplitis croceipes*, had previously been found to use associative learning to locate host insects. Applying this basic knowledge, food-deprived wasps were provided with a sucrose-water reward in the presence of specific odor concentrations to train them to the target odor. Having succeeded at training wasps, ARS scientists at Tifton, Georgia, applied this knowledge to train wasps to identify boar taint compounds (skatole, androstenone, and indole). They found that the need for a threshold detection of chemicals, rather than simple detection of absence/presence, applies to many food quality issues as well, including the detection of spoilage or pest damage in crops or stored foods. Bennett Aerospace has licensed the patent for this work from ARS and the University of Georgia. Additional work and expanded applications are being explored by ARS and the Department of Defense.

Olson, D.M., Wäckers, F.L., and Haugen, J.E. 2012. Threshold detection of boar taint chemicals using parasitic wasps. Journal of Food Science 77:356-361.

Wäckers, F., Olson, D., Rains, G., Lundby, F., and Haugen, J. 2011. Boar taint detection using parasitoid biosensors. Journal of Food Science 76:541-547.

***Crop and non-crop colonization of stink bug pests in farmscapes*.** Stink bugs cause economic damage in corn, cotton, soybean, pecan, peach, and vegetables in southeastern Coastal Plain farmscapes, which are composed of multiple fields of different crops whose edges interface with woodland habitats, usually located within 20 to 25 feet of the crop. The bugs damage the fruit, reducing yield, and carry at least one pathogen in cotton. Many growers in the region are using herbicide applications and bulldozing non-crop host plants in woodland habitats to eliminate or reduce populations of stink bugs in their crops. Knowledge of the importance of spatiotemporal distribution and dispersal of stink bugs in farmscapes is therefore critical for developing management strategies to control these pests, which require one or two aerial applications of pesticide each year in cotton alone. ARS scientists in Tifton, Georgia, and University of Florida collaborators showed that chinaberry, black cherry, uncultivated pecan, mimosa, beggarweed, pokeweed, and elderberry occur in woodland habitats and are sources of stink bugs in corn, peanut, and cotton. Some of these weeds are introduced invasives, and some, such as elderberry, can be reduced by mowing. Studying these systems, the scientists showed that the driving force for dispersal of stink bugs in these farmscapes is the need for new food and oviposition sites. For the southern green stink bug (*Nezara viridula*) and the brown stink bug (*Euschistus servus*), nymphs and adults develop in peanuts and subsequently disperse into cotton at the crop-to-crop

interface where they aggregate while feeding on cotton bolls. Even though peanut is not a significant source for another stink bug, the green stink bug (*Chinavia hilaris*), adults still disperse into cotton at the interface and field edges to search for food. Colonization of stink bugs is highest at the peanut-cotton interface, and all stink bugs studied exhibit edge-mediated dispersal as they colonize cotton. Growers and other researchers are beginning to adopt and test trap cropping systems at field edges for stink bugs, including trap crops in conventional and organic cropping systems for the brown marmorated stink bug (*Halyomorpha halys*), a new invasive species. This is important for development of new management strategies that will prevent damage by stink bugs to cotton, especially since cotton is the last host crop during the season.

Tillman, P.G. 2013. Likelihood of stink bugs colonizing crops: A case study in southeastern farmscapes. Environmental Entomology 42:438-444.

Tillman, P.G. 2011. Influence of corn on stink bugs (Heteroptera: Pentatomidae) in subsequent crops. Environmental Entomology 40:1159-1176.

Tillman, P.G., Northfield, T.D., Mizell, R.F., and Riddle, T.C. 2009. Spatiotemporal patterns and dispersal of stink bugs (Heteroptera: Pentatomidae) in peanut-cotton farmscapes. Environmental Entomology 38:1038-1052.

Induction of novel defense compounds in corn by European corn borer and pathogens. The European corn borer and stalk rotting pathogens cause more than $1 billion in losses each year, yet plant resistance mechanisms against these threats remain poorly understood. To better understand corn stalk defenses, ARS scientists in Gainesville, Florida, used metabolic profiling to search for elevated metabolite levels induced by borer and pathogen attack. Using this approach, the researchers identified six related acidic diterpenoids, termed kauralexins, that exhibit significant antifungal and insect anti-feedant activity. Importantly, the gene An2, encoding an ent-copalyl diphosphate synthase, was strongly implicated in regulating the biosynthesis of these defense compounds. Although these compounds demonstrated significant anti-feedant properties upon ingestion, the borer development was not affected in short-term studies. This may explain why the borer can inflict severe damage to corn plants. When given little other choice of host plants in monoculture landscapes, it appears that the borer may overcome these inducible anti-feedants and continue developing on corn. In addition, a mutation in maize, named an2, that blocks all inducible kauralexin production in corn has been identified. Field trials have recently begun using an2 maize plants to better understand how this mutation alters interactions with insects and other organisms. Collaborative experiments are also being conducted with an industry partner using their commercial maize lines. The company is building on ARS research to develop commercial lines resistant to fungal pathogens and to the borer.

Dafoe, N.J., Huffaker, A., Vaughan, M.M., Duehl, A.J., Teal, P.E., and Schmelz, E.A. 2011. Rapidly induced chemical defenses in maize stems and their effects on short-term growth of *Ostrinia nubilalis*. Journal of Chemical Ecology 37:984-991.

Huffaker, A., Kaplan, F., Vaughan, M.V., Dafoe, N.J., Ni, X., Rocca, J.R., Alborn, H.T., Teal, P.E.A., and Schmelz, E.A. 2011. Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize. Plant Physiology 156:2082-2097.

Schmelz, E.A., Kaplan, F., Huffaker, A., Dafoe, N.J., Vaughan, M.M., Ni, X., Rocca, J.R., Alborn, H.T., and Teal, P.E.A. 2011. Identity, regulation, and activity of inducible diterpenoid phytoalexins in maize. Proceedings of the National Academy of Sciences of the United States 108:5455-5460.

A new class of insect compounds may elicit a defensive response in corn. Much work is needed to understand and exploit plant resistance mechanisms to protect plants from insects. ARS scientists in Gainesville, Florida, and an international team of scientists are studying a unique type of resistance—plants that elicit chemicals to attract insect parasites to kill pests feeding on foliage. The researchers discovered a new class of these compounds, called caeliferins, which were produced by oral secretions of the American grasshopper, *Schistocerca americana*, while feeding on corn. The researchers found that adding these compounds to damaged leaves of corn seedlings induced the production of volatile organic compounds that is comparable to the well-known response to volicitin and other fatty acid amides previously discovered by the same researchers. Elicitors, such as the caeliferins, have provided a tool for better understanding the multifaceted physiological ecology of insect and plant interaction. In follow-up work, the scientists are mapping the phytohormone-based activity of insect-produced elicitors, including caeliferins, as a powerful tool to aid in the study of these complex interactions. The pattern of and exposure to caeliferins may determine whether *S. americana* grasshoppers are solitary or gregarious. These compounds could potentially be used to control the locusts by interfering with their swarming behavior.

Schmelz. E.A., Engelberth, J., Alborn, H.T., Teal, P.E.A., and Tumlinson, J.H. 2009. Phytohormone-based activity mapping of insect herbivore-produced elicitors. Proceedings of the National Academy of Sciences of the United States 106:653–657.

Alborn H.T., Hansen T.V., Jones T.H., Bennett D.C., Tumlinson J.H., et al. 2007. Novel disulfoxy fatty acids from the American bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. Proceedings of the National Academy of Sciences of the United States 104(32):12976-12981.

Discovery and inhibition of biochemical signaling in insect immunity. Insects protect themselves from microbial infections, parasitic invasions, and wounding through robust innate immune reactions. One of the research frontiers in the broad area of insect immunity is work on understanding how biochemical signals that mediate and coordinate cellular immune functions are translated and integrated within defense cells. ARS scientists in Columbia, Missouri, in collaboration with scientists at Andong National University, Republic of Korea, and Kansas State University, demonstrated that prostaglandin signaling depends on enzymes responsible for prostaglandin synthesis and that molecular silencing of the genes encoding these enzymes impairs insect immune reactions to bacterial infection. They also showed that prostaglandin signaling is translated by specific cellular receptors. The researchers demonstrated that one cellular defense reaction—cell spreading—is mediated by a peptide that acts via prostaglandin signaling. On the understanding that virtually all pest insects receive and recover from bacterial infections during their juvenile life stages, this work forms the conceptual basis for enhancing efficacy of microbial biological control programs by crippling insect immunity. These findings are being used by researchers working to understand and manipulate insect immunity.

Shrestha, S., Stanley, D., and Kim, Y. 2011. PGE2 induces oenocytoid cell lysis via a G protein-coupled receptor in the beet armyworm, *Spodoptera exigua*. Journal of Insect Physiology 57:1568-1576.

Srikanth, K., Park, J., Stanley, D.W., and Kim, Y. 2011. Plasmatocyte-spreading peptide influences hemocyte behavior via eicosanoids. Archives of Insect Biochemistry and Physiology 78:145-160.

Shrestha, S., Park, Y., Stanley, D., and Kim,Y. 2010. Genes encoding phospholipases A2 mediate insect nodulation reactions to bacterial challenge. Journal of Insect Physiology 56:324-332.

Tunaz, H. and Stanley, D.W. 2009. An immunological axis of biocontrol: Infections in field-trapped insects. Naturwissenschaften 96:115-119.

Disrupting insect diapause to control pest insects. A critical life function of numerous pest insects is the dormant state known as diapause, which allows insects to survive winter and other adverse conditions. Entering and exiting diapause is hormonally controlled by an insect's neuropeptides. ARS scientists in College Station, Texas, in collaboration with Ohio State University scientists, developed stable versions of "Diapause Hormone" neuropeptides, which are much more active than the insect's neuropeptides. Unlike the native neuropeptide, two of these novel compounds can also prevent entry into pupal diapause or block its termination when administered at the preceding larval stage of the corn earworm, killing the insect. This discovery will be used to develop a novel and environmentally friendly strategy to control pest insects by disrupting diapause.

Zhang, Q., Nachman, R.J., Kaczmarek, K., Zabrocki, J., and Denlinger, D.L. 2011. Disruption of insect diapause using agonists and an antagonist of diapause hormone. Proceedings of the National Academy of Sciences of the United States 108:16922-16926.

Understanding Bt resistance in pink bollworm. Pink bollworm in the United States has remained susceptible to Cry1Ac in Bt-cotton for more than 15 years, although laboratory colonies have been selected with resistance to the toxin and field populations of pink bollworm in India have evolved resistance. Advances in managing Bt resistance and preserving this important insect control technology depend on an understanding of resistance mechanisms and the genetic basis for resistance. ARS researchers in Maricopa, Arizona, with collaborators at the University of Arizona, have identified four mutations to a cadherin (a cell adhesion protein) that confers Bt resistance in the pink bollworm, including one mutation involving a mobile DNA element, or "jumping gene." These findings implicate changes in the cadherin gene as the predominant mechanism of Bt resistance in pink bollworm and provide the tools necessary to anticipate and monitor for resistance in the field. These molecular sampling tools greatly enhance the ability of researchers to detect and measure shifts in resistant gene frequencies in the field. This proactive approach to understanding resistance mechanisms and developing practical detection tools is a major step in sustained use of Bt-cotton in the United States.

Fabrick, J.A., Mathew, L.G., Tabashnik, B.E., and Li, X. 2011. Insertion of an intact CR1 retrotransposon in a cadherin gene linked with Bt resistance in the pink bollworm, *Pectinophora gossypiella*. Insect Molecular Biology 20(5):651-665.

Fabrick, J.A. 2010. Insights into the genetics and molecular mechanisms of pink bollworm resistance to Cry toxins. Southwestern Entomologist 35(3):431-435.

Carrière, Y., Showalter, A.M., Fabrick, J.A., Sollome, J., Ellers-Kirk, C., and Tabashnik, B.E. 2009. Cadherin gene expression and effects of Bt resistance on sperm transfer in pink bollworm. Journal of Insect Physiology 55(11):1058-1064.

Fabrick, J.A., Forlow Jech, L., and Henneberry, T.J. 2009. Novel pink bollworm resistance to the Bt toxin Cry1Ac: Effects on mating, oviposition, larval development, and survival. Journal of Insect Science 9:24.

Gassmann, A.J., Fabrick, J.A., Sisterson, M.S., Hannon, E.R., Stock, S.P., Carrière, Y., and Tabashnik, B.E. 2009. Effects of pink bollworm resistance to *Bacillus thuringiensis* on phenoloxidase activity and susceptibility to entomopathogenic nematodes. Journal of Economic Entomology 102(3):1224-1232.

Host utilization patterns enhance risk assessments of Bt resistance. More than 70 percent of cotton in the United States is planted to Bt-cotton varieties. In the southern United States, where insecticide-resistant lepidopteran pests threaten profitable production, more than 95 percent of the crop is planted to Bt varieties, primarily those expressing multiple Bt toxins. ARS scientists in Stoneville, Mississippi, and collaborators initiated a multi-state project to measure the host plant origin of bollworms colonizing Bt-cotton. A natural refuge for Bt-cottons expressing multiple toxins was approved by the Environmental Protection Agency (EPA) based in part on results of this project. The natural refuge concept was widely supported by the cotton industry, and most cotton farmers currently use the natural refuge option to comply with EPA resistance management requirements. Economic farm-level benefits of the natural refuge have been estimated to be as great as $22 to $26 per acre.

Allen, K.C. and Luttrell, R.G. 2011. Temporal and spatial distribution of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) moths in pheromone traps across agricultural landscapes in Arkansas. Journal of Entomological Science 46:269-283.

Head, G., Jackson, R.E., Adamczyk, J.J., Bradley, J.R., Van Duyn, J.W., Gore, J., Hardee, D.D., Leonard, B.R., Luttrell, R.G., Ruberson, J., Mullins, W., Orth, R.G., Sivasupramaniam, S., and Voth, R. 2010. Spatial and temporal variability in host use by *Helicoverpa zea* as measured by analyses of stable carbon isotope ratios and gossypol resides. Journal of Applied Ecology 47:583-592.

Allen, K.C. and R.G. Luttrell. 2009. Spatial and temporal distribution of heliothines and tarnished plant bugs across the landscape of large Arkansas farms. Crop Protection 28:722-727.

Jackson, R.E., Bradley, J. R., Van Duyn, J., Leonard, B.R., Allen, K.C., Luttrell, R., Ruberson, J., Adamczyk, J., Gore, J., Hardee, D.D., Voth, R., Sivasupramaniam, S., Mullins, J.W., and Head, G. 2008. Regional assessment of *Helicoverpa zea* populations on cotton and non-cotton crop hosts. Entomologia Experimentalis et Applicata 126:89-106.

Sequencing the western corn rootworm genome. Western corn rootworm is often called the "billion dollar insect" due to costs associated with reductions in corn yields and field control measures. An international effort coordinated by ARS scientists in Ames, Iowa, and researchers at the University of Illinois has begun to sequence the western corn rootworm genome, which holds promise for improved management options for corn producers in the United States. The scientists estimated the genome at 2.6 billion base pairs, almost the size of the human genome,

and have sequenced representative regions. Results indicate that genes occupy only about 9 percent of the genome, whereas other regions are composed of repeated and mobile DNA sequences, positioned within and between gene coding regions. One sequenced region of the genome contained the gene responsible for cyclodiene insecticide resistance, an evolved trait associated with insecticide chemistries used in the 1960s and 1970s. DNA markers were developed to estimate the distribution of corn rootworm resistance to cyclodienes in the United States, illustrating the potential application of markers to study changes in insect population genetics. Several single nucleotide polymorphism (SNP) markers were identified in the western corn rootworm sequence data using computational methods. A panel of over 1,500 candidate SNPs were evaluated and used to construct a high-density genetic linkage map. Other markers were used to characterize gene flow between populations. This was difficult to do previously because of less-sensitive genetic marker systems, and it is especially important now that resistance of the rootworm to transgenic Bt-corn has developed in the field, requiring the use of insecticides.

Coates, B.S., Alves A., Walden, K., French, B.W. Miller, N.J., Abel, C.A., Sappington, T.W., Robertson, H.M., and Siegfried, B.D. 2012. Distribution of genes and repetitive elements in the *Diabrotica virgifera* genome estimated using BAC sequencing. Journal of Biomedicine and Biotechnology Article ID 604076, 9 pages. DOI:10.1155/2012/604076.

French, B.W., Coates, B.S., and Sappington, T.W. 2012. Inheritance of extended diapause traits in the Northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae). Journal of Applied Entomology DOI:10.1111/j.1439-0418.2012.01751.x.

Coates, B.S., Sumerford, D.V., Kim, K.S., Miller, N.J., Sappington, T.W., Siegfried, B.D., and Lewis, L.C. 2009. Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. Journal of Heredity 100:556–564.

Development of western corn rootworm colonies with resistance to Bt-corn. The study and development of laboratory colonies of corn rootworms resistant to insecticidal transgenic corn help researchers understand how such resistance occurs and how to manage the resistance once it is present in field populations. Laboratory colonies of western corn rootworm were established from survivors of transgenic Bt-corn developed to control rootworms. The insecticidal proteins used for selection in the laboratory were engineered by seed companies from similar natural proteins, then expressed in corn. Colonies used in the studies included a selected colony (from adults isolated and grown on transgenic corn) and a control colony (from adults isolated and grown on conventional corn not expressing insecticidal proteins) for each of the transgenic corn types targeting rootworms that are registered for commercial use (including Cry3Bb1, mCry3A, Cry34/35Ab1, and eCry3.1Ab proteins). Laboratory-selected resistance developed in western corn rootworm populations to all insecticidal proteins currently registered for corn rootworm management. Further study of these colonies will facilitate an understanding of the mechanisms and genetics of resistance and allow researchers to explore potential cross-resistance among different Bt events.

Meihls, L.N., Higdon, M.L., Ellersieck, M.R., Tabashnik, B. E., and Hibbard, B.E. 2012. Greenhouse-selected resistance to Cry3Bb1-producing corn in three western corn rootworm populations. PloS ONE 7:e51055.

Meihls, L.N., Higdon, M.L., Ellersieck, M., and Hibbard, B.E. 2011. Selection for resistance to mCry3A expressing transgenic corn in western corn rootworm. Journal of Economic Entomology 104:1045-1054.

Meihls, L.N., Higdon, M.L., Siegfried, B.D., Miller, N.J., Sappington, T.W., Ellersieck, M.R., Spencer, T.A., and Hibbard, B.E. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. Proceedings of the National Academy of Sciences of the United States 105:19177-19182.

Potent new aphicides based on novel mode of action. Many aphids are resistant to insecticides, and research is needed to find other methods of control to protect crops. ARS scientists in College Station, Texas, in collaboration with scientists in Belgium, have developed a novel strategy for control of aphid populations based on stabilized versions of internal hormones known as neuropeptides. These hormones regulate critical life processes in aphids and other pest insects, but are rapidly inactivated by the insects' internal enzymes. The stabilized neuropeptide versions ("mimics") are resistant to inactivation by the enzymes and demonstrate high aphicidal effects following treatment. The new aphicides operate through a novel and very selective mechanism. The new agents match or exceed the potency of several aphicides that are used commercially. This discovery will be used to develop environmentally friendly strategies to control pest aphids based on their own chemical signals.

Nachman, R.J., Hamshou, M., Kaczmarek, K., Zabrocki, J., and Smagghe, G. 2012. Biostable and PEG-polymer conjugated insect pyrokinin neuropeptides demonstrate antifeedant activity and induce high mortality in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidae). Peptides 34:266-273.

Nachman, R.J., Mahdian, K., Nassel, D.R., Isaac, R.E., Pryor, N.W., and Smagghe, G. 2011. Biostable multi-Aib analogs of tachykinin-related peptides demonstrate potent oral aphicidal activity in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidae). Peptides 32:587-594.

Smagghe, G., Mahdian, K., Zubrzak, P., and Nachman, R.J. 2010. Antifeedant activity and high mortality in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidae) induced by biostable insect kinin analogs. Peptides 31:498-505.

PROBLEM STATEMENT IIB: *Control*

ARS research under this Problem Statement focused on the applied aspects of controlling pests of food and fiber crops. In this 5-year period, significant accomplishments were made toward the identification and deployment of insect-pest natural enemies, discovery of botanical extracts with insecticidal activities, and development of host plant resistance traits. ARS scientists also developed cultural control strategies for managing weeds and made progress toward developing organic bioherbicides.

Weeds

Population-based weed management reduces need for herbicides. Weeds continue to limit crop production, and herbicide-resistant weeds raise further problems for weed management. Producers of grain crops (e.g., wheat, corn, sunflower, and sorghum) in the Great Plains have

expressed the need for alternative weed management options that rely less on herbicides. ARS scientists in Brookings, South Dakota, developed a population-based weed management system that disrupts weeds in Great Plains croplands. Components of the system included rotating crops to inhibit certain weeds, diseases, and insects; using strategies that favor growth of crops over weeds (e.g., increasing crop seeding rates, narrowing row spacing, and targeting fertilizer application directly to the crop seed); and retaining crop residues in the ground by not tilling. This system significantly decreased the number of weed seeds produced in crop fields, while exposing them to temperature extremes and dry conditions by keeping the weed seeds closer to the soil surface. To determine which insects were the main consumers of seeds in this cropping system, ARS scientists developed a new technique to mark seeds using specific proteins, followed by gut analysis of insects to determine which species had consumed the marked seeds. The researchers discovered that seeds were reduced on average by 70 percent (green foxtail), 29 percent (lambsquarters), and 36 percent (pigweed) over a 5-day period. Use of a population-based approach to weed management is now being widely adopted by grain producers in no-till crop systems in the Great Plains. Surveys of Great Plains producers showed that use of the population-based weed management system resulted in 50 percent fewer herbicide applications. Producers reported cost savings of between $15 and $25 an acre, which enabled producers to rotate with different crops, thus accruing additional benefits, including suppression of pests and diseases and enhanced soil quality.

Lundgren, J.G., Saska, P., and Honěk, A. 2013. Molecular approach to describing a seed-based food web: the post-dispersal granivore community of an invasive plant. Ecology and Evolution 3:1642-1652.

Lundgren, J.G. and Harwood, J.D. 2012. Functional responses to food diversity in soil ecosystems: the effect of seed availability on the feeding behavior of facultative granivores. Journal of Entomological Science 47(2):160-176.

Anderson, R.L. 2010. A rotational framework to reduce weed density in organic systems. Renewable Agriculture and Food Systems 25:189-195.

Anderson, R.L. 2008. Rotation design and no-till: keys for pest management in the Great Plains. Weed Science 56:141-145.

Organic rotational no-till soybean production requires a multi-tactical weed management approach. Organic grains are in high demand, but traditional tillage-based organic soybean production practices have restricted the ability of producers to expand. This is due to the high labor requirements of weed management and to no-till farmers' unwillingness to adopt tillage due to the risks of lowering soil quality and an increase susceptibility to erosion. ARS scientists in Beltsville, Maryland, with their university collaborators in the mid-Atlantic region, have elucidated key principles involved in the benefits of using cover crop mulches (cereal rye, in this case) for weed management in an organic rotational no-till soybean system. The scientists developed a strategy for using cultural management practices (fertility, establishment method, planting and termination timing, and seeding rate) to manage cover crops for optimal biomass production and weed suppression. The strategy provides decision support tools to farmers that are based on their existing base soil fertility, crop rotations, and equipment constraints to best achieve successful production of soybeans using a cover crop-based, rotational no-till system.

This strategy is being circulated by peer-reviewed publications, extension bulletins, Web sites, and grower meeting presentations and/or materials and in production guides circulated at universities. Adoption by farmers is being observed throughout the eastern United States.

Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R., and Shumway, D.L. 2011. Timing of cover crop management effects on weed suppression in no-till planted soybean using a roller-crimper. Weed Science 59:380-389.

Ryan, M.R., Curran, W.S., Grantham, A.M., Hunsberger, L.K., Mirsky, S.B., Mortensen, D.A., Nord, E.A., and Wilson, D.O. 2011. Effects of seeding rate and poultry litter on weed suppression from a rolled cereal rye cover crop. Weed Science 59:438-444.

Ryan, M.R., Mirsky, S.B., Mortensen, D.A., Teasdale, J.R., and Curran, W.S. 2011. Potential synergistic effects of cereal rye biomass and soybean planting density on weed suppression. Weed Science 59:238-246.

Better timing of fertilizer applications reduces need for herbicides in rice fields. In recent years, fertilizers have been most frequently applied on the soil surface prior to flooding rice fields, thereby dissolving the inorganic phosphorus in the water and leading to excessive algal growth, especially by the cyanobacterium *Nostoc spongiaeforme*. These algae blooms form large mats that can smother rice seedlings or cause them to dislodge. ARS scientists in Davis, California, demonstrated that the proliferation of algae blooms in rice fields was caused by high levels of inorganic phosphorus fertilizer that was applied in rice-field water. The scientists found that rice fields receiving surface-applied phosphate fertilizer had 4 to 8 times more algal/cyanobacterial biomass and 3 to 11 times higher concentrations of soluble reactive phosphate than when phosphate was incorporated or delayed. The scientists, along with colleagues from the University of California, Davis, showed that economic yields were sustained by delaying phosphorous application about 1 month after rice-field flooding. These new practices have been adopted by rice growers and have significantly reduced algal growth in rice fields, as well as the need to apply costly algaecides.

Spencer, D.F. and Linquist, B.A. 2013. Reducing rice field algae and cyanobacteria abundance by altering phosphorus fertilizer applications. Journal of Paddy and Water Environment On-line. V.11(41).

Lundy, M.E., Spencer, D.F., Van Kessel, C., Hill, J.E., and Lindquist, B.A. 2012. Managing phosphorus fertilizer to reduce algae, maintain water quality, and sustain yields in water-seeded rice. Field Crops Research 131:81-87.

Discovering phytotoxins with new modes of action for weed management. No new herbicide modes of action have been introduced in over 20 years, yet ever-increasing numbers of weeds have evolved resistance to herbicides. Additionally, organic farmers have no economical means of weed management. To help cope with these problems, ARS scientists in Oxford, Mississippi, discovered several new natural compounds, or phytotoxins, with new modes of action that have potential as bioherbicides. Among these is ascaulitoxin, a potent toxin from a fungal weed biological control agent (*Ascochyta caulina*) that was shown to have a new mode of action involved with amino acid transport. This research has also elucidated the role of allelochemicals in the success of invasive weed species and uncovered new phytotoxins from two highly invasive weeds, congongrass and musk thistle. Pest management companies are consulting the

researchers regarding herbicide mode-of-action research projects, and the researchers have intiatied a cooperative research agreement with an industry partner for development of bioherbicides.

Cantrell, C.L., Dayan, F.E., and Duke, S.O. 2012. Natural products as sources for new pesticides. Journal of Natural Products 75:1231-1242.

Duke, S.O. 2012. Why have no new herbicide modes of action appeared in recent years? Pest Management Science 68:505-512.

Dayan, F.E., Cantrell, C.L., and Duke, S.O. 2009. Natural products in crop protection. Bioorganic & Medicinal Chemistry 17:4022-4034.

Prediction and mitigation of bioenergy crop invasion. Sustainable production of bioenergy crops, which supports a diversified U.S. energy portfolio, must include strategies to prevent the escape and invasive spread of bioenergy crop species, yet such strategies have not been integrated. ARS scientists in Urbana, Illinois, and collaborators demonstrated that a multi-tiered screening approach offers robust predictions of the invasive potential of plant species being considered for bioenergy production. Pre-introduction screening tools such as the Weed Risk Assessment (WRA) survey can successfully be paired with post-introduction field studies of plant population dynamics (with survival, reproduction, growth, and dispersal as parameters) to limit unintentional use of invasive plant species for bioenergy. The scientists compared WRA and projections of population growth rate with field measurements of invasiveness in introduced habitats. For those crop species deemed low risk, the field studies provided data to support best management practices proposed by the scientists for mitigating invasion risk. Proposed best management practices include siting bioenergy plantations on flat ground away from riparian areas, creating a 20-foot mowed buffer zone around bioenergy plantations to assist monitoring, and avoiding the use of *Miscanthus* varieties with fertile seeds. In 2011, these best management practices were used by the USDA Farm Service Agency, Natural Resource Conservation Service, and ARS partners in developing conservation guidelines for the safe management of *Miscanthus x giganteus* (a tall warm season grass, native to Asia) to protect adjoining natural areas on two different pilot projects of the Biomass Conversion Assistance Program, affecting 100,000 acres of farmland.

Matlaga, D., Schutte, B.J., and Davis, A.S. 2012. Age-dependent population dynamics of the bioenergy crop *Miscanthus x giganteus* in Illinois. Invasive Plant Science and Management 5:238-248.

Matlaga, D.P., Quinn, L.D., Stewart, R., and Davis, A.S. 2012. Light response of native and introduced Miscanthus sinensis seedlings. Invasive Plant Science and Management 5:363-374.

Quinn, L.D., Matlaga, D.P., Davis, A.S., and Stewart, R. 2011. Evaluating the influence of wind speed on caryopsis dispersal of *Miscanthus sinensis* and *Miscanthus x. giganteus*. Invasive Plant Science and Management 4:142-150.

Davis, A.S., Cousens, R.D., Hill, J., Mack, R.N., Simberloff, D., and Raghu, S. 2010. Screening bioenergy feedstock crops to mitigate invasion risk. Frontiers in Ecology and the Environment 8:533-539.

Manuka oil, a green weed management product with pre-emergence systemic herbicidal activity. Few green products are available for weed control and most are essential oils with burn-down effects that require farmers to apply them multiple times at relatively concentrated solutions (10 percent or more). ARS scientists in Oxford, Mississippi, have discovered that manuka oil has both pre- and post-emergent herbicidal activity, which is particularly potent against weedy grasses. This essential oil is rich in natural β-triketones that inhibit a key enzyme in the photosynthetic process, inhibiting plant growth and acting as a natural herbicide. Manuka oil can be applied as an herbicide either to leaves or to soils to inhibit growth. This research has received the interest of industry and the work has been expanded under the terms of a cooperative research agreement with Marrone BioInnovations, which is evaluating the potential synergism of the natural essential oil in combination with their commercial products.

Owens, D.K., Nanayakkara, N.P.D., and Dayan, F.E. 2013. In planta mechanism of action of leptospermone: Impact of its physico-chemical properties on uptake, translocation, and metabolism. Journal of Chemical Ecology 39:262-270.

Dayan, F.E., Singh, N., McCurdy, C., Godfrey, C.A., Larsen, L., Weavers, R.T., Van Klink, J.W., and Perry, N.B. 2009. β-triketone inhibitors of plant *p*-hydroxyphenylpyruvate dioxygenase: Modeling and comparative molecular field analysis of their interactions. Journal of Agricultural and Food Chemistry 57:5194–5200.

A fungal bioherbicide to control herbicide-resistant weeds. The extensive use of glyphosate on glyphosate-resistant crops has contributed to an increased incidence of herbicide resistance in weeds. Currently, few cost-effective alternatives are available to control such species. Palmer amaranth is one of the major weeds that has evolved resistance to glyphosate. ARS scientists in Stoneville, Mississippi, examined the efficacy of the bioherbicidal fungus *Myrothecium verrucaria* on whole plants and on excised leaf bioassays of glyphosate-resistant and glyphosate-susceptible Palmer amaranth plants. Generally, injury was directly proportional to the *M. verrucaria* concentration applied to the fungal mycelium, with glyphosate-resistant and glyphosate-susceptible plant leaves being equally sensitive to the *M. verrucaria* phytotoxic effects, as measured by reduction of chlorophyll content. Similar effects occurred on whole plants challenged by *M. verrucaria* spray applications to foliage. In young (2-week-old) plants, nearly 100 percent mortality occurred 72 hours after treatment. Older plants were more tolerant to the bioherbicide, but chlorosis and reduction of growth were typical symptoms. Results demonstrate that under greenhouse/laboratory conditions, *M. verrucaria* can control both glyphosate-resistant and glyphosate-susceptible Palmer amaranth seedlings, suggesting that this bioherbicide might be a candidate for use against this economically important weed. These research results suggest that *M. verrucaria* could be valuable potential alternative and more sustainable weed control method for researchers studying weed management systems for herbicide-resistant weeds. The scientists are also furthering their research on herbicide interactions with combinations of *M. verrucaria* with glyphosate for controlling kudzu, redvine, and trumpetcreeper.

Hoagland, R.E., Teaster, N.D., and Boyette, C.D. 2013. Bioherbicidal effects of *Myrothecium verrucaria* on glyphosate resistant and-susceptible Palmer amaranth biotypes. Allelopathy Journal 31(2):367-376.

Weaver, M.A., Boyette, C.D., and Hoagland, R.E. 2012. Bioherbicidal activity from washed spores of *Myrothecium verrucaria*. World Journal of Microbiology and Biotechnology 28:1941-1946.

Weaver, M.A., Hoagland, R.E., Boyette, C.D., and Zablotowicz, R.M. 2009. Macrocyclic trichothecene production and sporulation by a biological control strain of *Myrothecium verrucaria* is regulated by cultural conditions. World Mycotoxin Journal 2:35-43.

Synergistic interaction of herbicides for kudzu control. Kudzu, a perennial leguminous vine native to eastern Asia introduced into the United States in the late 1800s, now invades the United States from Florida to New York and is expanding westward to central Oklahoma and Texas, causing hundreds of millions of dollars of losses per year. This aggressive weed is very difficult to control using synthetic herbicides and is an over-wintering host of pathogenic Asian soybean rust. ARS scientists in Stoneville, Mississippi, found that glyphosate mixed with another herbicide, an acetolactate synthase inhibitor such as trifloxysulfuron or pyrithiobac, could control kudzu. Even with much lower application rates, the rate of growth inhibition was faster and stronger than for either product applied alone. The mixture of these herbicides provides greater and more rapid control at a much reduced cost to landowners.

Molin, W.T. and Lyn, M.E. 2012. Methods for controlling weeds including kudzu. U.S. Patent and Trademark Office, Patent no. 8,338,333, December 2012.

Insects

Attractants for brown marmorated stink bug identified. The brown marmorated stink bug is an invasive insect pest that has spread to 39 states. It causes severe injury to fruits, vegetables, and field crops and is a serious nuisance pest for homeowners and businesses. A season-long attractant is needed to detect and monitor brown marmorated stink bug population densities and to make informed management decisions. ARS scientists in Beltsville, Maryland, have confirmed that the brown marmorated stink bug is attracted to methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT), a pheromone of a different Asian stink bug species, and the researchers have developed and commercialized a new synthesis of this compound. In cooperation with ARS scientists in Kearneysville, West Virginia, they also discovered the true male-produced aggregation pheromone of the stink bug and confirmed in field trials that it is attractive to male and female adult and immature bugs. The pheromone was developed into a commercially friendly version that has been transferred to the private sector. The pheromone has proven potent and suitable for monitoring the brown marmorated stink bug, and it is now being considered for attract-and-kill or other management technologies to limit insecticide inputs into agroecosystems. ARS scientists in Beltsville also discovered that the performance of the brown marmorated stink bug pheromone could be enhanced (synergized) by MDT, providing a superior lure for a season-long monitoring. A patent application has been filed on discovery of the brown marmorated stink bug attractants.

Leskey, T.C., Wright, S.E., Short, B.D., and Khrimian, A. 2012. Development of behaviorally based monitoring tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) in commercial tree fruit orchards. Journal of Entomological Science 47:76-85.

Khrimian, A., Zhang, A., Leskey, T., Aldrich, J., and Weber, D. 2011. Compositions and methods to attract the Brown Marmorated Stink Bug, *Halyomorpha halys*. U.S. Patent and Trademark Office, Provisional Patent D.N. 0154-11, December 2011.

Khrimian, A., Shearer, P.W., Zhang, A., Hamilton, G.C., and Aldrich, J.R. 2008. Field trapping of the invasive brown marmorated stink bug, *Halyomorpha halys*, with geometric isomers of methyl 2,4,6-decatrienoate. Journal of Agricultural and Food Chemistry 56:197-203.

Aldrich, J.R., Khrimian, A., and Camp, M.J. 2007. Methyl 2,4,6-decatrienoates attract stink bugs and tachinid parasitoids. Journal of Chemical Ecology 33:801-815.

Pink hibiscus mealybug pheromone licensed. The pink hibiscus mealybug is an invasive pest that was accidentally introduced into the United States from Asia in 1984. It attacks a broad range of crops, forest trees, and ornamental plants and is estimated to cause upwards of $750 million in damage annually. Conventional insecticides are relatively ineffective against the mealybug because of its protective outer layer of wax. ARS scientists from Beltsville, Maryland, have made significant inroads to developing a pheromone strategy to trap, detect, monitor, and potentially control this pest. They found that two components (one being a novel cyclobutanoid monoterpene) were the principal constituents of a pheromone blend produced by the virgin female. Use of this pheromone as a detection tool was confirmed in tests in ornamental greenhouses, nursery gardens, teak plantations, parota trees, mango tree orchards, and guanabana tree orchards. By using this attractant, scientists were able to map the regional distribution of the pink hibiscus mealybug, aid early detection of new infestations, and allow timely release of biological control agents or application of other management strategies to effectively control the pest. APHIS has adopted this strategy to detect and monitor the spread of the pink hibiscus mealybug from potentially infested nursery stock to 36 states in the United States and to facilitate control and eradication programs against the mealybug in Puerto Rico, California, and Florida. Importantly, the researchers also found that encrytid wasp parasites (*Anagyrus kamali* and *Gyramusoidae indica*) of the pink hibiscus mealybug are not attracted to the pheromone source and that biological control of mealybug was therefore not disrupted in ornamental plantings. APHIS is now using the pheromone blend to chart the effectiveness of these parasites on mealybug populations. Also, since high concentrations of the pheromone were shown to repel the pink hibiscus mealybug, a mating disruption strategy could be useful to lower infestations and potentially eradicate the pest. ARS granted a license to an industry partner in South Carolina to produce and market the pheromone, which has been used by government agencies as a survey tool for the pink hibiscus mealybug in the United States, the Caribbean, and Mexico.

Zhang A. and Oliver J.E. 2011. Attractant pheromone for the male pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae). U.S. Patent and Trademark Office, Patent no. 8,062,651 B1.

González-Gaona, E., Sánchez-Martínez, G., Zhang, A., Lozano-Gutiérrez, J., and Carmona-Sosa, F. 2010. Validation of two pheromonal compounds for monitoring pink hibiscus mealybug in Mexico. Agrociencia 44(1):65-73.

Vitullo, J., Zhang, A., Mannion, C., and Bergh, C. 2009. Expression of feeding symptoms from pink hibiscus mealybug (Hemiptera: Pseudococcidae) by commercially important cultivars of hibiscus. Florida Entomologist 92(2):248-254.

Super lure for important insect predators and egg parasitoids developed for commercial production. Lacewings, which are excellent predators, are often abundant in farmscapes bordering crops, but they may not be present in adequate numbers for effective control of pests in crops. ARS scientists in Beltsville, Maryland, led research to identify the aggregation pheromone of the goldeneyed lacewing (*Chrysopa oculata*), an important insect predator that eats aphids and other agricultural pests. The availability of powerful pheromones attractive to wild female lacewings makes semiochemical manipulation of these insects for enhanced biological control feasible. Research and negotiations with a commercial partner resulted in a product based on iridodial attraction of wild lacewings to crops. The commercial product 'SuperLure' is composed of plant volatiles and semiochemicals to attract a spectrum of beneficial insects. It has been used in field trials with Jacob's Farm, the largest organic farmer in California. This accomplishment was recognized with a Federal Laboratory Consortium Award for Excellence in Technology Transfer for ARS.

Chauhan, K.R. and Weber, D.C. 2008. Ladybird (Coleoptera: Coccinellidae) tracks deter oviposition by the goldeneyed lacewing, *Chrysopa oculata*. Biocontrol Science and Technology 18(7):727-731.

Chauhan, K.R., Levi, V., Zhang. Q-H., and Aldrich, J.R. 2007. Female goldeneyed lacewings (Neuroptera: Chrysopidae) approach but seldom enter traps baited with the male-produced compound iridodial. Journal of Economic Entomology 100(6):1751-1755.

Dogwood borer pheromone discovered. The dogwood borer is an important pest of dogwood and causes severe economic problems on at least 19 species of fruit, nut, and ornamental trees in the eastern United States and Canada. Recently, the borer has become an increasingly important pest of apple trees, affecting more than 150,000 acres, and ornamental dogwood grown in nurseries. Chemical insecticides have been the primary tools for control of the borer. ARS scientists in Kearneysville, West Virginia, and Beltsville, Maryland, developed a pheromone-based strategy to detect, monitor, and manage this pest. They discovered that a three-component blend was produced by female borers and that a lure containing a trinary blend attracted males 28 times more than did a virgin female and about 400 times more than did the most effective commercially available lure. The scientists also found that a pheromone inhibitor could effectively disrupt the mate-finding behavior of males. The pheromone has been rapidly adopted for dogwood borer detection and monitoring, and the efficacy and cost of the mating disruption strategy is competitive with previous insecticide-based management systems. This has resulted in almost complete elimination of the need to apply chlorpyrifos, an organophosphorous insecticide. The feasibility of this technology was demonstrated through a cooperative research agreement with an industry partner. A U.S. patent application has been submitted, for which license agreements are in negotiation with two companies.

Frank, D., Zhang, A., Leskey, T., and Bergh, J.C. 2011. Electrophysiological response of female dogwood borer (Lepidoptera: Sesiidae) to apple volatile compounds. Journal of Entomological Science 46(3):204-215.

Zhang, A., Leskey, T., and Bergh, C. 2011. Female sex pheromone of the dogwood borer (DWB), *Synanthedon scitula*, and attraction inhibitor (antagonist). U.S. Patent and Trademark Office, Patent No. 7,943,159 B1. May 2011.

Bergh, J.C., Leskey, T.C., Walgenbach, J.F., Klingeman, W.E., Kain, D.P., and Zhang, A. 2009. Dogwood borer (Lepidoptera: Sesiidae) abundance and seasonal flight activity in apple orchards, urban landscapes and woodlands in five eastern States. Environmental Entomology 38(3):530-538.

Leskey, T.C., Bergh, C., Walgenbach, J.F., and Zhang, A. 2009. Evaluation of pheromone based management strategies for the dogwood borer (Lepidoptera: Sesiidae) in commercial apple orchards. Journal of Economic Entomology 102(3):1085-1093.

Chemical lure for trapping spotted wing drosophila. Spotted wing drosophila is a vinegar fly of Asian origin that causes damage to many fruit crops. It was found in California and the Pacific Northwest about 5 years ago and has recently spread to the Gulf Coast, the Carolinas, Wisconsin, and Michigan. For early detection of the spotted wing drosophila, improved trapping methods are needed that are more sensitive than the apple cider vinegar bait most often used for this purpose. ARS scientists in Wapato, Washington, and Poplarville, Mississippi, working with Oregon Department of Agriculture scientists, isolated and identified chemicals from a fermented food bait. The scientists discovered a combination of wine and vinegar that was potent as a bait. The volatile chemicals from these materials were then analyzed to determine which chemicals are detected by the fly. In a series of laboratory and field tests, a combination of acetic acid, ethanol, methionol, and acetoin was found to be far more attractive than the apple cider vinegar bait used in detection and monitoring programs. Additional benefits of the chemical blend are the ability to use a controlled release dispenser to maximize lure attractiveness and longevity, and reduced attractiveness of non-target insects, which reduces labor costs involved in trap checking and maintenance. Commercial lures that incorporate these chemicals are currently under development by two industry partners, and prototype lures are being tested. The chemical lure is also being studied by researchers at Oregon State University and Cornell University.

Cha, D.H., Adams, T., Landolt, P.J., and Rogg, H. 2012. Identification and field evaluation of wine and vinegar v olatiles that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. Journal of Chemical Ecology 38:1419-1431.

Landolt, P.J., Adams, T., and Rogg, H. 2012. Trapping spotted wing drosophila (Diptera: Drosophilidae) with vinegar, wine, acetic acid and ethanol. Journal of Applied Entomology 136:148-154.

Landolt, P.J., Adams, T., Davis, T.S., and Rogg, H. 2012. Spotted wing drosophila, *Drosophila suzukii* (Matumura) (Diptera: Tephritidiae) trapped with combinations of wines and vinegars. Florida Entomologist 95:326-332.

Practical uses of pear ester to improve management of codling moth. Pear ester, ethyl (*E, Z*)-2,4-decadienoate, is attractive to larvae and male and female adult codling moth, the primary insect pest of apple, pear, and walnut crops. ARS researchers in Wapato, Washington, along with university and industry researchers, developed a combination pheromone/pear ester attractant for the codling moth. The attractant is commercially available and is referred to as the Pherocon® CM-DA Combo Lure. It is now widely adopted by growers to monitor codling moths in orchards under mating disruption programs in the United States and other countries. The researchers also developed a sprayable microencapsulated formulation of the pear ester to synergize activity of insecticides. This allows growers to use reduced rates of insecticides and improve performance of more selective insecticides. Two products containing pear ester based on this work—the Cidetrak® DA-MEC sprayable material for larval control and the Cidetrak®

CM-DA Combo dispenser for mating disruption of adults—have been registered. These products are sold in Argentina, Chile, and Spain, and registration is anticipated in the United States for 2013.

Knight, A.L., Light, D.M., Chebny, V. 2012. Monitoring codling moth (Lepidoptera:Tortricidae) in orchards treated with pear ester and sex pheromone combo dispensers. Journal of Applied Entomology 137:214-224. DOI: 10.1111/j.1439-0418.2012.01715.x.

Knight, A.L., Steliniski, L.L., Hebert, V., Gut, L., Light, D., and Brunner, J. 2011. Evaluation of novel semiochemical dispensers simultaneously releasing pear ester and sex pheromone for mating disruption of codling moth (Lepidoptera: Tortricidae). Journal of Applied Entomology 136:79-86.

Knight, A.L. 2010. Improved monitoring of female codling moth (Lepidoptera:Tortricidae) with pear ester plus acetic acid in sex pheromone-treated orchards. Environmental Entomology 39(4):1283-1290.

Knight, A.L. 2010. Effect of sex pheromone and kairomone lures on catches of codling moth. Journal of Entomological Society of British Columbia 107:1-8.

Pear psylla pheromone and lure. Pear psylla is a major pest of pears in the United States, with psyllid nymphs feeding on the phloem in leaves and on developing fruit. The insect may decrease fruit quality by depositing honeydew on fruit or leaves that leads to growth of black sooty mold that ultimately reduces the photosynthetic potential of the plant. Heavy infestations, along with injection of toxins from the salivary glands into phloem, can cause premature leaf fall and, ultimately, loss of the crop. ARS scientists in Wapato, Washington, and researchers at the University of California have jointly identified a key component of the female psylla's sex pheromone, 13 methyl heptacosane (13-MeC27). The compound's discovery could give rise to lures for either monitoring the pest or mating disruption. The attractiveness of this compound to males was confirmed with an olfactometer, and field experiments in pear orchards demonstrated attraction of males. Under a patent application filed by ARS, the scientists intend to combine 13-MeC27 with other attractants to produce blends for use in pheromone dispensers, bait stations, or traps.

Brown, R.L., Landolt, P.J., Horton, D.R., and Zack, R.S. 2009. Attraction of *Cacopsylla pyricola* (Hemiptera: Psyllidae) to female psylla in pear orchards. Environmental Entomology 38:815-822.

Guedot, C., Horton, D.R., and Landolt, P.J. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female extracts and evidence of male-male repellency. Entomologia Experimentalis et Applicata 130:191-197.

Guedot, C., Millar, J.G., Horton, D.R., and Landolt, P.J. 2009. Identification of a sex attractant for male winterform pear psylla, *Cacopsylla pyricola*. Journal of Chemical Ecology 35:1437-1447.

Discovery and use of pheromones for control of major citrus pests. The *Diaprepes* root weevil and the Asiatic citrus leafminer are major pests of citrus in Florida. The root weevil is a serious pest of citrus nurseries, where adults feed on leaves of citrus and larvae girdle roots. Citrus leafminer larvae tunnel in the leaves and greatly exacerbate citrus canker. ARS scientists in Fort Pierce, Florida; Beltsville, Maryland; and Gainesville, Florida, developed new semiochemical-

based approaches for controling the two citrus pests. The scientists received patents for kairomones and a novel pheromone for the weevil that will be used in new lures, traps, and control methods. They are running on-farm validation trials in several Florida counties as they develop mating disruption systems for control of the leafminer and associated citrus canker disease. In collaboration with the University of Florida and a private company, the scientists also developed two new products based on insect pheromones for leafminer control. These pheromones are expected to contribute to reduced loss to leafminer damage, reduced incidence and severity of citrus canker, and reduced use of broad-spectrum insecticides through better detection of the pest and improved management decisions.

Lapointe, S.L., Alessandro, R., Robbins, P., Khrimian, A., Svatos, A., Dickens, J.C., Otálora-Luna, F. Kaplan, F., and Teal, P. 2012. Identification and synthesis of a male-produced aggregation pheromone for the neotropical root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Journal of Chemical Ecology 38:408-417.

Dickens, J.C., Otalora-Luna, F., Hammock, J.A., Lapointe, S.L., and Alessandro, R.T. 2011. Attractants and repellents for the tropical root weevil *Diaprepes abbreviates*. U.S. Patent and Trademark Office, Patent no. 8,066,979 B1, November 2011.

Lapointe, S.L., Khrimian, A., Dickens, J.C., and Teal, P. 2011. Identification and synthesis of a male-produced pheromone for the Neotropical root weevil *Diaprepes abbrevatus* (Coleoptera: Curculionidae). Provisional Applications for Patent reg. no. 36327; Docket No. 0125.10, approved December 2011.

Lapointe, S.L. and Stelinski, L.L. 2011. An applicator for high viscosity semiochemical products and intentional treatment gaps for mating disruption of *Phyllocnistis citrella*. Entomologia Experimentalis et Applicata 141:145-153.

Lapointe, S.L., Stelinksi, L.L., Evens, T.J., Niedz, R.P., Hall, D.G., and Mafra-Neto, A. 2009. Sensory imbalance as mechanism of mating disruption in the leafminer *Phyllocnistis citrella*: elucidation by multivariate geometric designs and response surface models. Journal of Chemical Ecology 35:896-903.

Identification of female sex pheromone of the navel orangeworm. The navel orangeworm is the principal insect pest of California almonds and an important pest of pistachios and walnuts. Synthetic sex pheromone lures are used extensively in U.S. agriculture to monitor the presence and prevalence of pest insects and to determine the need and timing of control measures. Although the primary female sex pheromone component for navel orangeworm was identified by ARS researchers decades ago, a full synthetic blend attractive to male moths had eluded identification. ARS scientists in Parlier, California, in collaboration with University of California researchers, identified three additional pheromone compounds that, when combined with the primary pheromone component, elicited male responsiveness equal to both female pheromone extracts in the laboratory and caged females in the field. Because both the major pheromone component and two of the minor components are extremely unstable, several years of additional research was needed to develop a formulation suitable for field use. Based on this research, a private company has recently initiated marketing of a pheromone lure that is stable for several weeks and equals females in their attractiveness to male moths in the field. The lure will be used in approximately 1.5 million acres of high-value nut crops with an annual farm-gate value in excess of $4 billion.

Kanno, H., Kuenen, L.P.S., Klingler, K.A., Millar J.G. and Cardé R.T. 2010. Attractiveness of a four-component pheromone blend to male navel orangeworm moths. Journal of Chemical Ecology 36:584–591.

Kuenen, L.P.S., Leal, W.S. Millar, J.G., Pesak, D.J., Parra-Pedrazzoli, A.L., and Zalom, F.G. 2010. Navel Orangeworm Pheromone Composition. U.S. Patent and Trademark Office, Patent no. 7,655,253 B2.

Kuenen, L.P.S., McElfresh, J. S., and Millar, J.G. 2010. Identification of critical secondary components of the sex pheromone of the navel orangeworm (Lepidoptera: Pyralidae). Journal of Economic Entomology 103(2):314-330.

Mating disruption for control of navel orangeworm. In addition to their use in monitoring, pheromones can be used in mating disruption. ARS scientists in Parlier, California, developed and demonstrated a practical system of mating disruption for control of navel orangeworm. Timed-release aerosol dispensers were found to be more efficacious than membrane dispensers. For large areas of contiguous mating disruption, mating disruption in combination with limited application of methoxyfenozide, a reduced-risk insecticide, resulted in lower damage than either method alone. Economic analysis showed that mating disruption provided a net positive benefit on average over the entire 5 years. The research has expanded options for managing damage from the navel orangeworm. Over the period of this project, commercial mating disruption went from non-existent to nearly 40,000 acres of almonds and other tree nuts. Near-complete elimination of insecticide for navel orangeworm was documented in 2,500 acres—although actual reduction in insecticide usage is probably greater—and resulted in a net savings of $18 per acre and a major reduction in the use of conventional insecticides.

Higbee, B.S. and Burks, C.S. 2008. Effects of mating disruption treatments on navel orangeworm (Lepidoptera: Pyralidae) sexual communication and damage in almonds and pistachios. Journal of Economic Entomology 101(5):1633-1642.

New fruit fly dispenser deployed in California. Early detection of introduced pests is needed so that prompt deployment of eradication tactics can be implemented to keep the new pests from establishing. Trimedlure, methyl eugenol, and cue-lure traps are used to detect early introductions of fruit flies for immediate eradication. Recently, a novel solid wafer was developed by ARS scientists in Hilo, Hawaii, and an industry partner that incorporates trimedlure with raspberry ketone, methyl eugenol, and dichlorvos (Mallet TMR solid wafer). In survey trials near Kona, Hawaii, captures of Mediterranean fruit fly, oriental fruit fly, and melon fly with the Mallet TMR wafers were equal to those for the standard Trimedlure, methyl eugenol, and cue-lure traps used in Florida and California. The scientists, in cooperation with researchers from the University of California, are evaluating the new TMR wafers in typical citrus growing areas of California. Two series of trials were conducted, one in conditions representative of summer (August–September) and one representative of winter (January–March) conditions in California. The TMR Mallet solid wafers with DDVP may be more cost-effective, convenient, and safer to handle than current liquid lure and insecticide formulations used for detection programs in California. The scientists plan to develop cost-benefit analyses of Mallet TMR wafer use vs. standard trapping systems.

Vargas, R.I., Souder, S., Mackey, B., Cook, P.J., Morse, J.G., and Stark, J.D. 2012. Field trials of solid triple lure (trimedlure, methyl eugenol, raspberry ketone, and DDVP) dispensers for detection and male annihilation of *Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in Hawaii. Journal of Economic Entomology 105:1557-1565.

Methods to increase shelf life of fungus-based biopesticides developed. Acceptance and use of mycoinsecticides is constrained by low temperature storage requirements, especially in the tropics. In collaboration with scientists from Cornell University and EMBRAPA (Brazil), ARS scientists in Ithaca, New York, determined optimal conditions for storage of the common insect pathogenic fungus *Beauveria bassiana* at high temperatures in an anerobic environment (hermetically sealed packages with oxygen/moisture scavengers). Investigations focused on the composition of the storage container atmosphere, particularly with respect to moisture and oxygen content and the speed of equilibration. Longest survival of *B. bassiana* spores (conidia) was observed under exceedingly dry, anaerobic conditions supporting a minimal rate of metabolism. The optimal moisture level was lower than previously reported for storage in the presence of oxygen. Combined conditions of low moisture/low oxygen preserved conidial viability for more than 1 year at 40º C, the longest survival of conidia of an insect pathogenic fungus yet reported at such a high temperature. EMBRAPA applied for a Brazilian patent on this technology in 2010 and submitted applications the following year to the United States and 20 other countries. These findings are leading to development of formulation and packaging methods that will enhance shelf life and potency of fungus–based biopesticides worldwide.

Faria, M., Hotchkiss, J.H., and Wraight, S.P. 2012. Application of modified atmosphere packaging (gas flushing and active packaging) for extending the shelf life of *Beauveria bassiana* conidia at high temperatures. Biological Control 61(1):78-88.

Faria, M., Hotchkiss, J.H., and Wraight, S.P. 2010. Embalagem em atmosfera modificada para aumento da vida-de-prateleira de fungos entomopatogênicos. Brazilian patent applied for July 2010.

Methodologies and strategies for control of ambrosia beetles with entomopathogenic fungi. The ambrosia beetle, *Xylosandrus germanus* (Coleoptera: Curculionidae), an invasive pest with a wide host range, has become a serious pest of orchards and nurseries in the eastern and midwestern United States. The insect is difficult to control using conventional insecticides because applications must be made prior to adult emergence. This results in repeated applications and increased risks associated with exposure of non-target organisms. To test fungi for biocontrol of the beetle, ARS scientists in Ithaca, New York, first optimized conditions for laboratory rearing of the insect on a sawdust-based diet. This increased availability of insects for research facilitated additional studies on microbial control and other pest ecology and control projects. Using the laboratory rearing system, the scientists evaluated biocontrol potential of the insect pathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum* against adult ambrosia beetle females, including measurements of impacts of fungal treatments on beetle reproduction. *B. bassiana* and *M. brunneum* were virulent. Inoculation of adult females resulted in reduced gallery formation and brood production (with up to 100 percent of progeny infected). All life stages of the beetle were susceptible to infection, further illustrating the potential of these agents to impact beetle populations in the field.

Castrillo, L.A., Griggs, M.H., and Vandenberg, J.V. 2012. Brood production by *Xylosandrus germanus* (Coleoptera: Curculionidae) and growth of its fungal symbiont on artificial diet based on sawdust of different tree species. Environmental Entomology 41:822-827.

Castrillo, L.A., Griggs, M.H., Ranger, C.M., Reding, M.E., and Vandenberg, J.V. 2011. Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) against adult *Xylosandrus germanus* (Coleoptera: Curculionidae) and impact on brood. Biological Control 58:121-126.

New biological control agents established against tarnished plant bug. The tarnished plant bug (*Lygus* bug) injures many crops throughout the United States, and native natural enemies have little impact on its populations. ARS researchers in Newark, Delaware, previously introduced a European natural enemy of this pest, the parasitic braconid wasp *Peristenus digoneutis,* which established in the northeastern United States following field releases from 1979 to1988. Although this parasitic wasp has reduced tarnished plant bug populations and its importance as a pest, it did not establish in hotter areas of the United States. Foreign exploration by the researchers subsequently identified new geographic populations of tarnished plant bug natural enemies in southern Europe and northern Africa, and several are becoming widespread in central and coastal California. One of the natural enemies, *Peristenus relictus,* has been credited with having a significantly negative affect on populations of tarnished plant bug that invade strawberries on California's central coast. Surveys show a continual downward trend in tarnished plant bug populations and concurrent increases in levels of parasitism. Partners in this project include the California and New Jersey State Departments of Agriculture and scientists from the University of California. ARS foreign exploration for new natural enemies of tarnished plant bug has been supported in part by funding from the California Department of Food and Agriculture and the California Strawberry Commission.

Day, W.H. and Hoelmer, K. 2012. Impact of the introduced parasitoid *Peristenus digoneutis* (Hymenoptera: Braconidae) on tarnished plant bug (Hemiptera: Miridae) infesting strawberries in northwestern New Jersey, USA. Biocontrol Science and Technology 22:975-979.

Pickett C.H., Swezey, S.L., Nieto, D.J., Bryer, J.A., Erlandson, M., Goulet, H., and Schwartz, M.D. 2009. Colonization and establishment of *Peristenus relictus* (Hymenoptera: Braconidae) for control of *Lygus* spp. (Hemiptera: Miridae) in strawberries on the California Central Coast. Biological Control 49:27–37.

Bon, M.C., Hoelmer, K., Coutinot, D., and Ramualde, N. 2008. PCR-based rapid diagnosis of parasitism of *Lygus* spp. (Hemiptera: Miridae) by *Peristenus relictus* (Hymenoptera: Braconidae). Biocontrol Science and Technology 18:509-521.

Pickett, C.H., Rodriguez, R., Coutinot, D., Hoelmer, K.A., Kuhlmann, U., Goulet, H., and Schwartz, M. 2007. Establishment of *Peristenus* spp. (Hymenoptera: Braconidae) in California for the control of *Lygus* spp. Biocontrol Science and Technology 17: 261-272.

New methods to commercially produce fungi that kill insect pests and ticks. The stability of microbial products for insect control is problematic, particulalry in environmentally challenging soil environments. ARS researchers in Peoria, Illinois, developed the first methods to grow and commercially produce high concentrations of stable forms of the bioinsecticidal fungus *Metarhizium anisopliae,* which can be used to infect and kill a number of soil-dwelling insect pests such as Japanese beetle grubs, wireworms, rootworms, and root maggots. Subsequently,

the researchers were able to use these methods to produce the same fungus to kill lesser mealworm larvae and adults in commercial poultry operations. Recently, the ARS team adapted these methods to produce a related fungus, *M. brunneum*, to kill deer ticks that carry Lyme disease. This entomopathogenic fungus, when used in combination with a repellant known as nootkatone, provides a one-two punch for tick control. The team had previously formulated nootkatone for tick control in collaboration with scientists at the Connecticut Agricultural Experiment Station. In recent work with Brazilian scientists, a granular formulation of *Metarhizium* microslerotia is showing promise for tick control. The United States Golf Association has partnered with the ARS scientists to explore potential applications to control turf pests. Work is ongoing with scientists at Cornell University to control the Asian longhorned beetle with microsclerotial granules. The technology was licensed to a private sector partner in 2012. The commercialization of these biocontrol agents will provide homeowners, farmers, and land managers an effective non-chemical control for soil-dwelling insect pests and ticks.

Behle, R.W., Jackson, M.A., and Flor-Weiler, L.B. 2013. Efficacy of a granular formulation containing *Metarhizium brunneum* F52 (Hypocreales: Clavicipitaceae) microsclerotia against nymphs of *Ixodes scapularis* (Acari: Ixoididae). Journal of Economic Entomology 106:57-63.

Petzold-Maxwell, J.L., Jaronski, S.T., Clifton, E.H., Dunbar, M.W., Jackson, M.A., and Gassman, A.J. 2013. Interactions among Bt maize, entomopathogens, and rootworm species (Coleoptera: Chrysomelidae) in the field: effects on survival, yield and root injury. Journal of Economic Entomology 106(2):622-632.]

Bharadwaj, A., Stafford III, K.C., and Behle, R.W. 2012. Efficacy and environmental persistence of nootkatone for the control of the blacklegged tick (Acari: Ixodidae) in residential landscapes. Journal of Medical Entomology 49:1035-1044.

Jackson, M.A. and Jaronski, S.T. 2012. Evaluation of pilot-scale fermentation and stabilization processes for microsclerotia of the entomopathogenic fungus *Metarhizium anisopliae.* Journal of Biocontrol Science and Technology 22:915-930.

Flor-Weiler, L.B., Behle, R.W., and Stafford III, K.C. 2011. Susceptibility of four tick species, *Amblyomma americanum, Dermacentor variabilis, Ixodes scapularis, and Rhipicephalus sanguineus* (Acari: Ixodidae), to nootkatone from essential oil of grapefruit. Journal of Medical Entomology 48:322-326.

New biocompatible foams deliver living microbial agents for insect pest control. Many pest insects hide in locations that cannot be reached with conventional insecticide sprays. Pest control operators commonly use foam spraying technologies to deliver insecticides to these difficult-to-reach insect habitats. Unfortunately, the chemicals typically used in pesticide foams are detrimental to most microbial biological control agents, reducing their efficacy or even preventing their use. In addition, most foaming agents cannot be used in organic farming operations due to the nature of their chemical composition. ARS researchers in Peoria, Illinois, have developed and patented a natural, protein-based foaming agent technology that is compatible with living microbial agents and safe for use in organic pest control. This technology has been successfully used to treat a number of insect pests, including several moth and beetle pests of fruit trees as well as Formosan subterranean termites. Formosan subterranean termite control, damage, and prevention costs in the United States exceed $1 billon a year. Although natural termiticidal microbial agents have been identified, their effectiveness has been limited by

how and where they can be applied due to the termites' subterranean nature. The technology developed by the Peoria team overcomes those problems and provides a mechanism for expanded use of microbial control for termite control and organic crop systems. This research was featured on the History Channel television show "Modern Marvels" in 2009.

Dunlap, C.A., Jackson, M.A., and Wright, M.S. 2012. Compositions of keratin hydrolysates and microbes for pest control applications. U.S. Patent and Trademark Office, Patent no. 8,263,526 B2.

Jackson, M.A., Dunlap, C.A., and Jaronski, S.T. 2010. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. Biocontrol 55:129-145.

Wright, M.S., Connick Jr., W.J., and Jackson, M.A. 2008. Use of *Paecilomyces* spp. as pathogenic agents against subterranean termites. U.S. Patent and Trademark Office, Patent no. 7,390,480 B2.

Dunlap, C.A., Jackson, M.A., and Wright, M.S. 2007. A foam formulation of *Paecilomyces fumosoroseus*, an entomopathogenic biocontrol agent. Biocontrol Science Technology 17:513-523.

Black pecan aphid control using a plant bioregulator. Use of conventional insecticides for management of the tree-defoliating black pecan aphid negatively affects natural enemies of insects and often triggers secondary pest management problems in orchards. ARS scientists in Byron and Tifton, Georgia, determined how black pecan aphid-elicted leaf chlorosis affects development of aphid individuals and populations. The scientists found that certain plant bioregulators, gibberallic acid and forchlorfenuron, alone and in combination, prevent black pecan aphids from triggering leaf chlorosis. The result is greater aphid mortality through retarded insect development and eventual starvation and an extension of canopy health and longevity. This discovery is being used for black pecan aphid management, with about 5,000 acres of pecans in Georgia being treated during 2011 and 2012 with gibberellic acid as part of an aphid management strategy. The scientists believe this strategy is likely to work against other aphid species on crops where aphids elicit chlorotic-type feeding.

Cottrell, T.E., Wood, B.W., and Ni, X. 2010. Application of plant growth regulators mitigates chlorotic foliar injury by the black pecan aphid (Hemiptera: Aphididae). Pest Management Science 66:1236-1242.

Cottrell, T.E., Ni, X., and Wood, B.W. 2009. Chlorotic feeding injury by the black pecan aphid (Hemiptera: Aphididae) to pecan foliage promotes aphid settling and nymphal development. Environmental Entomology 38:411-416.

New species of biological control agents approved for field release against soybean aphid. The invasive Asian soybean aphid has become a major pest in the United States, with 18 percent of the 77 million acres of soybean planted in 2012 needing to be treated with insecticides, mostly to control the aphid. Biological control using indigenous Asian parasitoids specific to soybean aphid is a promising approach for control of this pest. ARS researchers in Newark, Delaware, with assistance from the ARS Sino-American Biological Control Laboratory and other Asian cooperators, searched in Asia for key natural enemies of the soybean aphid and discovered at least five natural enemy species new to science. Host-range research by the ARS scientists and cooperators at the University of Minnesota identified several of these species as effective and

safe for introduction. Petitions for field releases of three of these natural enemies (*Binodoxys* and *Aphelinus* parasitic wasps) have received regulatory approval from APHIS, and releases have been initiated with cooperators in several north-central States. Successful introduction of these natural enemies will enhance biological control of this invasive aphid, thus helping to reduce damage and pesticide inputs and preserve the environment.

Hopper, K.R., Woolley, J.B., Hoelmer, K.A., Wu, K., and Lee, S. 2012. An identification key to species in the *mali* complex of *Aphelinus* (Hymenoptera, Chalcidoidea) with descriptions of three new species. Journal of Hymenoptera Research 26:73-96.

Heimpel, G.E., Frelich L.E., Landis D.A., Hopper K.R., Hoelmer KA, Sezen Z, Asplen M.K., and Wu, K-M. 2010. European buckthorn and Asian soybean aphid as components of an extensive invasional meltdown in North America. Biological Invasions 12:2913-29331.

Stary, P., Tomanovic, Ž., Rakhshan, E., Hoelmer, K.A., Kavallieratos, N.G., Yu, J.-J., Wang, M.-Q., and Heimpel, G.E. 2010. A new species of Lysiphlebus Förster 1862 (Hymenoptera: Braconidae, Aphidiinae) attacking soybean aphid, *Aphis glycines* Matsumura (Hem., Aphididae) from China. Journal of Hymenoptera Research 19:184-191.

Desneux, N., Barta, R.J., Hoelmer, K.A., Hopper, K.R., and Heimpel, G.E. 2009. Behavioral and physiological determinants of host specificity in an aphid parasitoid. Oecologia 160:387-398.

Desneux, N., Starỳ, P., Delebecque, C.J., Gariepy, T.D., Barta, R.J., Hoelmer, K.A., and Heimpel, G.E. 2009. Cryptic species of parasitoids attacking the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in Asia: *Binodoxys communis* Gahan and *Binodoxys koreanus* Stary sp. n. (Hymenoptera: Braconidae: Aphidiinae). Annals of the Entomological Society of America 102:925-936.

Wyckhuys, K.A.G., Stone, L., Desneux, N., Hoelmer, K., Hopper, K.R., and Heimpel, G.E. 2008. Parasitism of the soybean aphid *Aphis glycines* by *Binodoxys communis*: the role of aphid defensive behavior and parasitoid reproductive performance. Bulletin of Entomological Research 98:361-370.

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Integrated management of disease-vectoring thrips. Thrips and thrips-vectored diseases are among the most serious pests of vegetable, ornamental, and agronomic crops. ARS scientists in Tallahassee, Florida, with collaborators at the University of Florida and the Chinese Academy of Agricultural Sciences, developed an integrated management program for thrips in tomatoes and peppers that includes realistic economic thresholds, conservation of non-pest thrips that out-compete pests, ultraviolet reflective mulches and essential plant oils that repel thrips from crops, biopesticides (such as entomophagous fungi), and vertical integration of thrips management with management systems for other pests, including whiteflies and several lepidopterans. These recommendations were disseminated to the public through the University of Florida Web site and are being adopted by many growers in the southeastern United States.

Reitz, S.R. and J. Funderburk. 2012. Management Strategies for Western Flower Thrips and the Role of Insecticides, pp. 355-384, In: Insecticides - Pest Engineering, Farzana Perveen (Ed.), In Tech, Rijeka, Croatia.

Funderburk, J., Reitz, S., Olson, S., Stansly, P., Smith, H., McAvoy, G., Demirozer, O., Snodgrass, C., Paret, M., and Leppla, N. 2011. Managing thrips and tospoviruses in tomato, ENY-859. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Sobhy, I.S., Sarhan, A.A., Shoukry, A.A., El-Kady, G.A., Mandour, N.S., Funderburk, J., Reitz, S., Stansly, P., Schuster, D., Nuessly, G., and Leppla, N. 2009. Managing Thrips in Pepper and Eggplant. University of Florida, IFAS Extension. Publication ENY-658.

Reitz, S.R., Maiorino, G., Ritchie, L., Olson, S., Sprenkel, R., Crescenzi, A., and Momol, M.T. 2008. The effects of plant essential oils and particle films on tomato spotted wilt and thrips in tomatoes. Plant Disease 92:878-886.

Novel application of environmentally friendly nematodes controls key peach pests. Peach trees suffer major damage from the plum curculio, which damages the fruit; tree borer larvae, which damage below-ground portions of the tree; and the lesser peachtree borer, which attacks the cambium and inner bark of the trunk and scaffold limbs. Scheduled insecticide sprays are used for control of all three pests. ARS researchers in Byron, Georgia, collaborated with scientists from the University of Georgia and the University of Florida to determine whether two entomopathogenic nematodes could be used to control all three insects using different application scenarios for the different pests. In replicated field trials, one soil application of *Steinernema riobrave* suppressed 78 to 100 percent of plum curculio larva. For the peachtree borer, just one springtime soil application of *S. carpocapsae* suppressed larval infestations by an average of 88 percent. In another field trial, three soil applications during the peachtree borer's fall egg-laying season reduced infestations to levels equal to those of standard insecticides. Controlling the lesser peachtree borer is more difficult because they attack trees above the ground, where the beneficial nematodes are subject to desiccation and UV radiation. To overcome this problem, *S. carpocapsae* was applied using a sprayable fire-gel, a novel formulation that enhances survival and longevity of beneficial nematodes and thus their ability to control the borer and other above-ground pests. Studies indicated 70 to 100 percent suppression of the lesser peachtree borer when the entomopathogenic nematodes were applied in the sprayable fire-gel. Collectively, this research showed that nematode applications are economically feasible in comparison with conventional insecticides. The scientists are now expanding their work to optimize applications and formulations of the nematodes in peach tree orchards and to evaluate their use as a control method in apple orchards (in cooperation with ARS researchers in Kearneysville, West Virginia). Research and application stemming from this technology are also being pursued for use of beneficial nematodes in other commodities such as greenhouse crops and for enhancing the efficacy of other bio-pesticides.

Shapiro Ilan, D.I., Mizell, R.F., Cottrell, T.E., and Horton, D.L. 2008. Control of plum curculio, *Conotrachelus nenuphar* with entomopathogenic nematodes: effects of application timing, alternate host plant, and nematode strain. Biological Control 44:207-215.

Mass production methods of an environmentally friendly bio-pesticide. A major barrier to wider adoption of beneficial entomopathogenic nematodes in biological pest control is the cost of production. ARS researchers in Byron, Georgia, and Stoneville, Mississippi, collaborated with a commercial company to increase the efficiency of producing beneficial nematodes in insect hosts. The team improved all stages of production, including nematode strain enhancement, insect rearing, and nematode inoculation, harvest, and packaging procedures. Improved methodology enables greater production efficiency of insects used to grow the nematodes through enhanced nutritive diets and mechanization of equipment. These advances greatly reduce the unit cost of production, especially labor cost, and are enabling a 200 to 300 percent increase in production efficiency and sales. This is triggering additional research to

improve production technology. The research also led to two issued and two pending patents and a Federal Laboratory Consortium Award for Excellence in Technology Transfer in 2013. The industry partner in the research has adopted all of the technologies, and others in the industry are expressing interest.

Shapiro Ilan, D.I., Rojas, M.G., Morales-Ramos, J.A., and Tedders, W.L. 2012. Optimization of a host diet for in vivo production of entomopathogenic nematodes. Journal of Nematology 44:264–273.

Morales-Ramos, J.A., Rojas, M.G., Shapiro Ilan, D.I., and Tedders, W.L. 2011. Nutrient regulation in *Tenebrio molitor* (Coleoptera: Tenebrionidae): SELF-selection of two diet components by larvae and impact on fitness. Environmental Entomology 40:1285-1294.

Shapiro Ilan, D.I., Cottrell, T.E., Mizell, R.F., Horton, D L, Behle, R.W., and Dunlap, C.A. 2010. Efficacy of *Steinernema carpocapsae* for control of the lesser peachtree borer, *Synanthedon pictipes*: Improved above-ground suppression with a novel gel application. Biological Control 54:23–28.

Shapiro Ilan, D.I., Morales-Ramos, J.A., Rojas, M.G., and Tedders, W.L. 2010. Effects of a novel entomopathogenic nematode–infected host formulation on cadaver integrity, nematode yield, and suppression of *Diaprepes abbreviatus* and *Aethina tumida* under controlled conditions. Journal of Invertebrate Pathology 103:103-108.

Shapiro Ilan, D.I., Cottrell, T.E., Mizell III, R.F., Horton, D.L., and Davis, J. 2009. A novel approach to biological control with entomopathogenic nematodes: Prophylactic control of the peachtree borer, *Synanthedon exitiosa.* Biological Control 48:259-263.

Sweetpotato germplasm identified with multiple pest resistance. One of the best ways to achieve cost-effective pest control is to breed for resistant crops. ARS scientists in Charleston, South Carolina, in collaboration with researchers at Louisiana State University and North Carolina State University, identified sources of germplasm in sweetpotato with resistance to multiple pests and diseases. Using recurrent mass selection techniques, resistance factors were incorporated into breeding materials. These new sweetpotato genotypes were assessed for quality, including yield, shape, color, texture, dry matter, and taste, and for resistance to root knot nematodes and soil insect pests such as flea beetle larvae, wireworms, sweetpotato weevils, and *Diabrotica* larvae. Resin glycosides and other chemical components from the periderm and cortex of insect-resistant sweetpotato clones were found to negatively affect survival, development, and fecundity of insect pests and nematodes and to negatively affect competing weeds (allelopathic effects). This work has led to several sweetpotato germplasm releases with inclusion in the ARS National Germplasm System in Griffin, Georgia. Information on resistance factors and resistant germplasm are being used by growers and public researchers to more effectively utilize available plant genetic resources in enhancing pest and disease resistance in sweetpotato.

Jackson, D.M. and Harrison Jr., H.F. 2013. Insect resistance in traditional and heirloom sweetpotato varieties. Journal of Economic Entomology 106(3):1456-62.

Jackson, D.M., Harrison Jr., H.F., and Ryan-Bohac, J. 2012. Insect resistance in sweetpotato plant introduction accessions. Journal of Economic Entomology 105:651-658.

Jackson, D.M. and Bohac, J.R. 2007. Resistance of sweetpotato genotypes to adult *Diabrotica* beetles. Journal of Economic Entomology 100:566-572.

Novel bacterium with activity against insects commercialized. The biopesticide Bt was discovered decades ago and is effective against lepidopteran insects as well as some beetles and mosquitoes. However, most piercing-sucking plant feeders, are not killed by Bt strains. ARS scientists in Beltsville, Maryland, discovered and described a new soil-dwelling bacterium, *Chromobacterium subtsugae*, that has minimal impact on non-target insects but kills many key pest insects, including Colorado potato beetle, small hive beetle, southern corn rootworm, diamondback moth, and piercing-sucking insects such as stink bugs, Asian citrus psyllid, whiteflies, and mites. This new biological pesticide represents a major contribution to biological control, providing two mechanisms for toxin exposure: ingestion and contact. It is believed that a heat-stable toxin is involved in killing the insect because autoclaved bacteria also killed the insects. Furthermore, this organism appears to have multiple modes of action that may make it difficult for insects to develop resistance. *C. subtsugae* represents the first new insecticidal bacterium to receive EPA registration and approval in over 50 years. Two pest control companies licensed the patent for this organism in 2007, and the resulting biopesticide is currently sold in over 30 states as the commercially formulated product Grandevo®. This discovery and successful commercialization of a new insecticidal bacterium resulted in a Federal Laboratory Consortium Award for Excellence in Technology Transfer for ARS in 2013.

Martin, P.A.W and M.B. Blackburn. 2008. Characterization of the insecticidal activity of *Chromobacterium subtsugae.* Biopesticide International 4:102-109.

Martin, P.A W., Gundersen-Rindal, D., Blackburn, M., and Buyer, J. 2007. *Chromobacterium subtsugae* sp. nov., a novel beta - protobacterium toxic to Colorado potato beetle and other insect pests. International Journal Systematic and Evolutionary Microbiology 57:993-999.

Martin, P.A.W., Hirose, E., and Aldrich, J.R. 2007. Toxicity of *Chromobacterium subtsugae* to Southern Green Stink Bug and Corn Rootworm. Journal of Economic Entomology 100:680-684.

Martin, P.A.W., Shropshire, A., Gundersen-Rindal, D., and Blackburn, M.B. 2007. *Chromobacterium subtsugae* Sp. Nov. and Use for Control of Insect Pests. U.S. Patent and Trademark Office, Patent no. 7,244,607.

Baculoviruses with improved genetic characteristics selected for biocontrol. Baculoviruses are used as bioinsecticides in many cropping systems, but their use could be expanded if their speed of kill were enhanced to minimize crop losses from targeted insect pests. Identification and utilization of new baculovirus strains with optimal activity against insect pests requires detailed biological and genotypic data. Using molecular genetics approaches, ARS scientists in Beltsville, Maryland, identified and classified baculoviruses in approximately 200 virus samples from the insect virus collection at Beltsville and other sources. Complete genome sequences were determined to obtain detailed genetic information on a selection of these viruses. Bioassays were carried out to determine biological activities of selected wild-type and clonally derived isolates against significant important lepidopteran pests, including fall armyworm (*Spodoptera frugiperda)*, black cutworm, and species of the heliothine complex (*Helicoverpa zea*, *H. armigera*, and *Heliothis virescens*) and the cole crop pest complex (*Trichoplusia ni* and *Plutella xylostella*). The sequence data generated were used by a number of groups to better understand baculoviruses and identify samples for commercial development. Based on this work, a comprehensive study on the evolution and relationships of baculoviruses has been conducted at

the Institut de Recherche sur la Biologie de l'Insecte in Tours, France. Research utilizing the black cutworm baculovirus genome sequence is underway at a university in Germany in conjunction with a Swiss company to develop the black cutworm baculovirus as a biocontrol agent. In addition, data on fall armyworm baculoviruses have led to a cooperative research project with ARS scientists and an Austrailian company to develop an effective baculovirus-based biopesticide for use against infestations of fall armyworm. Interest in this virus is keen because it is a fast killer, with speed of kill found to be twice that of other known baculovirus strains in both the laboratory and under field conditions. This represents the first effort to commercialize a novel baculovirus insecticide for the United States market in more than a decade.

Breitenbach, J.E., El-Sheikh, E.A., Harrison, R.L., Rowley, D.L., Sparks, M.E., Gundersen-Rindal, D.E., and Popham, H.J.R. 2013. Determination and analysis of the genome sequence of *Spodoptera littoralis* multiple nucleopolyhedrovirus. Virus Research 171:194-208.

Behle R.W. and Popham H.J.R. 2012. Laboratory and field evaluations of the efficacy of a fast-killing baculovirus isolate from *Spodoptera frugiperda*. Journal of Invertebrate Pathology 109:194-200.

Harrison, R.L., Popham, H.J.R., Breitenbach, J.E., and Rowley, D.L. 2012. Genetic variation and virulence of *Autographa californica* multiple nucleopolyhedrovirus and *Trichoplusia ni* single nucleopolyhedrovirus isolates. Journal of Invertebrate Pathology 110:33-47.

Rowley, D.L., Popham H.J.R., and Harrison, R.L. 2011. Genetic variation and virulence of nucleopolyhedroviruses isolated worldwide from the heliothine pests *Helicoverpa armigera*, *Helicoverpa zea*, and *Heliothis virescens*. Journal of Invertebrate Pathology 107:112-126.

Harrison, R.L., Puttler, B., and Popham, H.J.R. 2008. Genomic sequence analysis of a fast-killing isolate of *Spodoptera frugiperda* multiple nucleopolyhedrovirus. Journal of General Virology 89(3):775-790.

Exploiting host finding and flight behavior of ambrosia beetles in ornamental nurseries.
Invasive ambrosia beetles, including *Xylosandrus* spp., are serious damaging pests in ornamental tree nurseries and are especially problematic in Atlantic, midwestern, and southeastern States. Growers rely on trunk sprays of insecticides to protect trees from attack by these beetles. However, synchronizing treatments with beetle activity is difficult because the beetles are very small and difficult to detect, and knowledge of their seasonal activity, host-selection behavior, and chemical ecology in nurseries is lacking. ARS scientists in Wooster, Ohio, in collaboration with researchers at Ohio State University, Tennessee State University, and Virginia Tech University, studied host-finding behavior, seasonal attack activity, and efficacy of insecticides for control of ambrosia beetles. The research demonstrated the significance of ethanol as an attractant for the beetles and the ability of physiological stress to predispose trees to attack. A push-pull strategy for managing ambrosia beetles in nurseries was developed based on this information, and on-site management practices are being adopted by growers to maintain host vigor and eliminate management practices that stress the trees. Monitoring techniques were refined based on observations that *Xylosandrus germanus* flight activity is related to temperature and these techniques can be used to forecast beetle activity and assist decisions on applying protective sprays. Initial flight activity was also associated with bloom sequence of woody ornamental nursery plants, suggesting that growers can use bloom sequence to time sprays.

Ranger, C.M., Reding, M.E., Schultz, P., and Oliver, J. 2013. Influence of flood-stress on ambrosia beetle (Coleoptera: Curculionidae, Scolytinae) host-selection and implications for their management in a changing climate. Agricultural and Forest Entomology 15:56-64.

Ranger, C.M., Tobin, P.C., Reding, M.E., Bray, A.M., Oliver, J.B., Schultz, P.B., Frank, S.D., and Persad, A. B. 2013. Interruption of the semiochemical-based attraction of ambrosia beetles to ethanol-baited traps and ethanol-injected trap trees by verbenone. Environmental Entomology 42:539-547.

Reding, M E., Ranger, C.M., Oliver, J.B., and Schultz, P.B. 2013. Monitoring attack and flight activity of *Xylosandrus* spp. (Coleoptera: Curculionidae: Scolytinae); the influence of temperature on activity. Journal of Economic Entomology 106:1780-1787.

Reding, M.E., Oliver, J., Schultz, P., Ranger, C.M., and Youssef, N. 2013. Ethanol injection of ornamental trees facilitates testing insecticide efficacy against ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Journal of Economic Entomology 106:289-298.

Ranger, C.M., Reding, M.E., Gandhi, K.J., Oliver, J.B., Schultz, P.B., Cañas, L., and Herms, D.A. 2011. Species dependent influence of (-) α pinene on attraction of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) to ethanol-baited traps in nursery agroecosystems. Journal of Economic Entomology 104:574-579.

Managing exotic scarabs and root weevils with botanical extracts and reduced-risk insecticides. Root-herbivory by exotic scarabs and root weevils causes serious damage in ornamental nurseries, and the management of exotic pests (including black vine weevil, Japanese beetle, and ambrosia beetles) costs the nursery industry more than $1 billion a year. ARS scientists in Wooster and Toledo, Ohio, in collaboration with researchers from several universities and industry, isolated and identified a compound in zonal geraniums (quisqualic acid) that causes paralysis of Japanese beetle adults following consumption of flower petals. The chemical basis for paralysis had remained unknown since the phenomenon was first described in the 1920s by J. J. Davis and C. H. Ballou. This is the first report of quisqualic acid in a plant other than *Quisqualis indica*. Zonal geraniums could potentially be used to develop a botanical formulation for controlling Japanese beetles and other insect pests and improve host plant resistance strategies.

Ranger, C.M., Reding, M.E., Oliver, J.B., Moyseenko, J.J., Youssef, N. and Krause, C.R. 2013. Acute toxicity of plant essential oils to scarab larvae (Coleoptera: Scarabaeidae) and their analysis by gas chromatography-mass spectrometry. Journal of Economic Entomology 106:159-167.

Ranger, C.M., Winter, R.E., Singh, M.E., Reding, M.E., Frantz, J., Locke, J.C., and Krause, C.R. 2011. A rare excitatory amino acid from flowers of zonal geranium responsible for paralyzing the Japanese Beetle. Proceedings of the National Academy of Sciences of the United States 108(4):1217-1221.

Ranger, C.M., Reding, M.E., Oliver, J.B., Moyseenko, J.J., Youssef, N.N. 2009. Toxicity of botanical formulations to nursery-infesting white grubs (Coleoptera: Scarabaeidae). Journal of Economic Entomology 102:304-308.

Integrated pest management of the sweetpotato whitefly. The sweetpotato whitefly represents a complex of cryptic species that cause major losses of yield and quality to a multitude of fruit, vegetable, and ornamental crops worldwide. Management of this pest complex is particularly difficult because of its mobility, high reproductive rate, wide host range, ability to vector diseases, and insecticide resistance. ARS scientists in Maricopa, Arizona, with University of Arizona collaborators, elucidated key factors critical to whitefly management. Preservation of natural enemies, particularly arthropod predators, was found to be essential to management of the whitefly, whereas manipulation of host quality (drought stressed, non-stressed) was not. Long-term field studies have shown that mortality inflicted by several exotic parasitoid species has not increased over that from the native parasitoids they displaced. New, selective insecticides (spiromesifen, spirotetramat, cyazypyr, and sulfoxaflor) were found to provide whitefly control with minimal impact on natural enemies, and a new experimental insecticide (pyrifluquinazon) was found to prevent whitefly transmission of Cucurbit yellows stunting disorder virus to cantaloupe. Insecticide studies also indicated that an integrated approach using a combination of field and laboratory assays was necessary to accurately evaluate insecticide efficacy and assess the rate of development of resistance. As part of an overall cotton IPM program, this research contributed to a 90-percent reduction in insecticide use and savings of nearly $400 million to Arizona cotton growers over the past 15 years. Research approaches and results have been applied by scientists and producers in Brazil, Mexico, Turkey, and Australia.

Carrière, Y., Ellers-Kirk, C., Hartfield, K., Larocque, G., DeGain, B., Dutilleul, P., Dennehy, T.J., Marsh, S.E., Crowder, D.W., Li, X., Ellsworth, P.C., Naranjo, S.E., Palumbo, J.C., Fournier, A., Antilla, L., and Tabashnik, B.E. 2012. Large-scale, spatially explicit test of the refuge strategy for delaying insecticide resistance. Proceedings of the National Academy of Sciences of the United States 109(3):775-780.

Dennehy, T.J., DeGain, B.A., Harpold, V.S., Zaborac, M., Morin, S., Fabrick, J.A., Nichols, R.L., Brown, J.K., Byrne, F.J., and Li, X. 2010. Extraordinary resistance to insecticides reveals exotic Q biotype of *Bemisia tabaci* in the New World. Journal of Economic Entomology 103(6):2174-2186.

Castle, S.J., Prabhaker, N., Henneberry, T.J., and Toscano, N.C. 2009. Host plant influences on susceptibility of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to insecticides. Bulletin of Entomological Research 99(3):263-273.

Naranjo, S.E. and Ellsworth, P.C. 2009. Fifty years of the integrated control concept: moving the model and implementation forward in Arizona. Pest Management Science 65(12):1267-1286.

Stage- and age-dependent injury to cotton by the western tarnished plant bug. Plant bugs currently represent the most important pest complex in U.S. cotton. The potential for crop injury by plant bugs is widely acknowledged, but efforts to define injury patterns in reference to insect age or developmental stage have yielded inconsistent and ambiguous results. A clearer understanding of stage- or age-dependent plant bug injury will be necessary to optimize economic thresholds and management strategies. ARS scientists in Shafter, California, devised methods to assess feeding behaviors and corresponding injury to cotton for plant bugs of different stages and age classes. Young, pre-reproductive adult bugs fed more and produced more plant injury than did older, reproductive adults. Large (fifth instar) nymphs fed similarly to young adults but produced more injury by virtue of their more frequent residence on fruiting

terminals. Smaller (third instar) nymphs fed more than other bug stages but produced less plant injury because of their small size and propensity to visit fewer flower buds. Results document previously unrecognized sources of variation in plant injury from plant bugs and represent the first repeatable demonstration of age- or stage-specific plant bug injury to cotton. The methodology and approaches used will be valuable to future efforts to quantify plant bug injury and provide researchers and plant breeders a means to efficiently screen cotton lines for resistance or tolerance. Information from this work was transferred to California growers and industry stakeholders and supports the recent incorporation of nymphal counts into treatment decisions for California cotton.

Cooper, W.R. and Spurgeon, D.W. 2012. Injury to cotton by adult *Lygus hesperus* (Hemiptera: Miridae) of different gender and reproductive states. Environmental Entomology 41(2):342-348.

Spurgeon, D.W. and Cooper, W.R. 2012. Temperature-dependent reproductive development of *Lygus hesperus* (Hemiptera: Miridae). Environmental Entomology 41(4):941-949.

Spotted wing drosophila management. Spotted wing drosophila is a new invasive pest in the mainland United States (2009) that attacks small fruits and stone fruits and causes serious economic damage because it attacks fruit that is still ripening. In laboratory and field studies, ARS scientists in Corvallis, Oregon, found fruits to be susceptible to infestation at the ripening coloration stage. Growers now use this coloration stage as a guideline to time their treatments and have eliminated one or two early-season sprays at a cost-savings of as much as $61an acre for materials and labor. The scientists also screened major pesticides in the laboratory and field and determined the duration of potency. Based on these data, growers have modified their management programs to use the most efficacious products at optimal intervals. In addition, the ARS scientists led a large trapping improvement project with other university researchers from seven States/provinces in North America that allowed them to identify the most effective traps and improve the physical features of the traps, such as greater entry area, bait surface area, side entry, and red/yellow color. A commercial company has marketed a red-colored trap using this information. The success of this project and service to the stakeholders were recognized with the Pacific Branch Entomological Society of America Team Award in 2012.

Lee, J.C., Burrack, H.J., Barrantes, L.D., Beers, E.H., Dreves, A.J., Hamby, K.A., Haviland, D.R., Isaacs, R., Richardson, T., Shearer, P., Stanley, C.A., Walsh, D.B., Walton, V.M., Zalom, F.G., and Bruck, D.J. 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. Journal of Economic Entomology 105:1350-1357.

Bruck, D.J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, J., Gerdeman, B., and Spitler, H. 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Management Science 67:1375-1385.

Lee, J.C., Bruck, D.J., Curry, H., Edwards, D., Haviland, D.R., Van Steenwyk, R.A., and Yorgey, B.M. 2011. The susceptibility of small fruits and cherries to spotted wing drosophila, *Drosophila suzukii*. Pest Management Science 67:1358-1367.

Black vine weevil management. The black vine weevil is a ubiquitous pest of nursery and fruit crops that feeds on plant roots. Nursery growers in the United States and Europe have limited management options, with only a few registered insecticides for black vine weevil control. ARS

scientists in Corvalis, Oregon, are investigating alternative management approaches, including *Metarhizium anisopliae,* a fungal entomopathogen. The scientists demonstrated long-term persistence and efficacy of Met52™ (*M. anisopliae* product registered for use in nursery crops) for black vine weevil control when the fungus was applied as a curative drench to soil-less potting media. The fungal treatment was also shown to be compatible with most fungicides when both were mixed in bulk soils. The scientists are exploring additional options to manage the weevil with the use of attractants. In collaboration with Dutch scientists, they found several key chemicals to attract the weevil that were sufficiently unique to warrant pursuit of a patent application. The researchers also found that pheromone traps made of fabric ruffles more effectively trapped beetles than did hard plastic traps because of the weevils' tendency to hide in plant leaves. These improved traps are being adopted and commercialized in Europe.

Van Tol, R., Griepink, F.C., and Bruck, D.J. 2011. Attractant compositions for weevils of the genus *Otiorhynchus* and uses thereof. U.S. Patent and Trademark Office, Patent application filed June,10, 2011.

Bruck, D.J. 2009. Impact of fungicides on *Metarhizium anisopliae* in the rhizosphere, bulk soil and *in vitro*. BioControl 54:597-606.

Bruck, D.J. 2007. Efficacy of *Metarhizium anisopliae* as a curative application for black vine weevil (*Otiorhynchus sulcatus*) infesting container-grown nursery crops. Journal of Environmental Horticulture 25:150-156.

Bruck, D.J. and Donahue, K.M. 2007. Persistence of *Metarhizium anisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container-grown ornamentals. Journal of Invertebrate Pathology 95:146-150.

***Suppression of tarnished plant bug with a Mississippi native strain of* Beauveria bassiana.** About a decade ago, ARS scientists in Stoneville, Mississippi, isolated a strain of *Beauveria bassiana*, strain NI8, from field collections of tarnished plant bug that had 10-fold more activity against Mississippi populations of the tarnished plant bug. Research has continued to improve production of NI8 and identify ecological niches where strategic use could suppress populations of the bug on an area-wide basis. Previous area-wide management of the tarnished plant bug by mowing and use of herbicides to destroy early-spring hosts demonstrated that insecticide use in cotton could be reduced by 50 percent when the cultural management practice was deployed on an area-wide basis. Increased corn acreage and early-production-system soybean have changed the agricultural landscape. Both crops have been documented as plant bug hosts, with spring and early-summer populations of tarnished plant bug now more widely dispersed across the landscape. Two potential deployment strategies have been identified: spraying of overwintering hosts with NI8 and spraying cotton with mixtures of NI8 and novaluron, an insect growth regulator, when plant bug populations first colonize cotton. The scientists found that efficacy of NI8 plus novaluron was as good or better than the traditional insecticides being used. Cage and field studies have confirmed suppression of tarnished plant bug when overwintering hosts are sprayed. Tests against key insect predators indicate that NI8 does not have higher activity than commercial strains on key insect predators and spiders. Effective suppression of the tarnished plant bug before they colonize cotton could dramatically improve grower profits and lessen the ecological impact of insecticides in the southern United States.

Ugine, T.A. 2012. Developmental time and age-specific life tables for *Lygus lineolaris* (Heteroptera: Mirdiae). Environmental Entomology 41:1-10.

Portilla, M., Streett, D., and G. Snodgrass. 2011. Effect of modification of the NI artificial diet on the biological fitness parameters of mass reared *Lygus hesperus* Knight (Hemiptera: Miridae). Journal of Insect Science 11:1-10.

Snodgrass, G. L., Jackson, R.E., Perera, O.P., Allen, C., and Luttrell, R.G. 2011. Utilization of tall goldenrod by the tarnished plant bug (Hemiptera: Miridae) in the production of overwintering adults and as a possible winter food source. Southwestern Entomologist 36:225-232.

Ugine, T.A. 2011. The effect of temperature and exposure to *Beauveria bassiana* on tarnished plant bugs *Lygus lineolaris* (Heteroptera: Miridae) population dynamics and the broader implications of treating insects with entomopathogenic fungi. Biological Control 59:373-383.

IPM programs in sweetpotato builds opportunities for small-scale farms in the Mississippi Delta. ARS scientists in Stoneville, Mississippi, have worked with small-scale farmers in the Mississippi Delta for more than a decade to improve IPM systems and increase quality and yield of sweetpotatoes. Studies have addressed the biology and ecology of important insect and nematode pests of sweetpotato and the development of environmentally friendly management systems. Cooperative projects with Alcorn State University, Mississippi State University, and Louisiana State University have provided a knowledge base for increasingly complex ecological, genetic, and physiological studies of sweetpotato. ARS researchers have developed a rating system to evaluate insect damage at harvest, refined insecticide selection and application methods, quantified the impact of crop rotation on risk of soil-borne pest attack in subsequent years, and benchmarked effective insect-management systems for grower comparisons. Yield of on-farm plots managed with the improved IPM system is comparable to that of large-scale production farms and about 40 percent higher than that measured for typical small-scale farms. Annual evaluations of varieties and comparisons of pest control options continue in on-farm field tests to encourage increased adoption of the new IPM systems.

Reed, J.T., Abel, C.A., and Adams, L.C. 2010. Comparison of three insect sampling methods in sweetpotato foliage in Mississippi. Journal of Entomological Science 45:11-128.

Reed, J.T., Shankle, M.W., Williams, M.R., and Burdine, W. 2010. Results of southern sweetpotato IPM project in Mississippi. Bulletin 1181, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, 14 pp.

Adams, L.C. and Johnson, C. 2007-2011. National sweetpotato collaborators variety trials, Summary of data USDA, ARS, SIMRU, Stoneville, MS. National Sweetpotato Collaborators Group Progress Report. 2007:33-34; 2008:31-32; 2009:36-37; 2010:34-35; 2011;42-43.

Wild rose habitat enhances biological control of apple leafrollers. Leafrollers are the larvae of a group of moths that feed on leaves and damage the fruits of many tree fruit varieties. Leafroller pests have many naturally occurring predators and parasites, but these generally do not prevent fruit damage because the insects enter orchards from surrounding habitats too late in the season or in low abundance. Growers need to use insecticides to control the leafrollers. ARS scientists in Wapato, Washington, with collaborators from Washington State University, showed

that plantings of wild rose adjacent to apple orchards can provide habitat for the reproduction of a beneficial parasitic wasp [*Colpoclypeus florus* (Eulophidae) of European origin], which will attack pest leafrollers in adjacent orchards. Since the creation and testing of this conservation/biological control approach throughout central Washington, apple growers with rose habitats are reducing or eliminating pesticide applications for leafrollers. In consultation with ARS scientists and with material help from NRCS, growers continue to add wild rose plantings for this purpose. More than 50 of these gardens (0.1 to 1 acre in size) have been planted around orchards in Washington. Based on these studies, NRCS expanded wildlife habitat enhancement projects in central Washington to include plantings of wild rose to support this beneficial leafroller parasite.

Pfannenstiel, R.S., Mackey, B.E., and Unruh, T.R. 2012. Leafroller parasitism across an orchard landscape in central Washington and effect of neighboring rose habitats on parasitism. Biological Control 62:152-161.

Unruh, T.R., Pfannenstiel, R.F., Peters, C., Brunner, J., and Jones, V.P. 2012. Parasitism of leafrollers in Washington fruit orchards is enhanced by perimeter plantings of rose and strawberry. Biological Control 62:162-172.

IPM tactics for the Asian citrus psyllid advanced. The Asian citrus psyllid is an important pest of citrus that transmits bacteria responsible for the serious disease of citrus known as Huanglongbing (HLB, or citrus greening disease). Since 2006, the disease has cost Florida's economy an estimated $3.6 billion in lost revenues and 6,611 jobs. ARS scientists in Fort Pierce, Florida, have made major advances toward improving psyllid management strategies through a number of different research efforts. They developed psyllid detection methods and sampling protocols that can be used by researchers, regulatory personnel, and growers. The scientists discovered volatiles collected from citrus leaves that may be useful for new or improved psyllid trapping and control methods. Traditional insecticides applied on a calendar schedule to control the Asian citrus psyllid were found to be largely ineffective and did not prevent newly planted trees from becoming infected with Huanglongbing. Alternatives to insecticides were advanced, including the use of two entomopathogenic fungi, a new psyllid-infecting virus, and new insect parasitoids, although the impact of the latter will continue to be negated to a large extent until the use of insecticides applied to conventional citrus is reduced. Citrus germplasm was identified with resistance to the psyllid, and traits responsible for this resistance may be useful in a traditional or transgenic breeding program aimed at reducing the incidence and spread of the psyllid and disease.

In additional ARS research on psyllid flight activity, it was shown that psyllid populations should be managed on an area-wide basis because the insect routinely disperses between citrus groves. Studies of the pest's physiology and genetics revealed new control opportunities. A new psyllid control tactic based on disruption of feeding was identified and patented. Target genes for an RNAi control tactic for the Asian citrus psyllid were identified and patented, and a cooperative research project was established with an industry partner to further develop RNAi tactics using these genes.

In yet another study in cooperation with ARS scientists in Albany, California; Weslaco, Texas; Poplarville, Mississippi; and Peoria, Illinois, the researchers determined the ability of one of the entomopathogenic fungi (*Isaria fumosorosea*) to contribute to integrated chemical-biological

control of the psyllid. Laboratory and greenhouse studies demonstrated that the insects can acquire fungal spores from coated cards baited with an attractive color and scent and that they can transmit fungus to other psyllids not exposed to cards. This creates practical options for alternative controls of this invasive pest of citrus.

Burand, J.P. and W.B. Hunter. 2013. RNAi: Future in insect management. Journal of Invertebrate Pathology 112(1):S68–S74. http://dx.doi.org/10.1016/j.jip.2012.07.012.

Hall, D.G., Gottwald, T.R., Stover, E., and Beattie, G.A.C. 2013. Evaluation of management programs for protecting young citrus plantings from huanglongbing. HortScience 48:330-337

Hall, D.G., Richardson, M.L., Ammar, E.D., and S.E. Halbert. 2013. Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), vector of citrus huanglongbing disease. Entomologia Experimentalis et Applicata 146:207-223

Mann, R.S., Rouseff, R., Smoot, J., Rao, C.N., Meyer, W.L., Lapointe, S.L., Robbins, P., Cha, D., Webster, F., Linn, C., Tiwari, S., and Stelinski, L.L. 2013. Chemical and behavioral analysis of the cuticular hydrocarbons from Asian citrus psyllid, *Diaphorina citri*. Insect Science 20:367-378.

Hunter, W.B., Avery, P.B., Pick, D., and Powell, C.A. 2011. Broad spectrum potential of *Isaria fumosorosea* on insect pests of citrus. Florida Entomologist 94:1051-1054.

Moran, P.J., Patt, J.M., Cabanillas, H.E., Adamczyk Jr., J.A., Jackson, M.A., Dunlap, C.A., Hunter, W.B., and Avery, P.B. 2011. Localized autoinoculation and dissemination of *Isaria fumosorosea* for control of the Asian citrus psyllid in south Texas. Subtropical Plant Science 63:23-35.

Development of banker plant systems for biological control of greenhouse pests. Whiteflies and thrips are serious economic pests of many vegetable and ornamental plants. ARS scientists in Fort Pierce, Florida, working in collaboration with the University of Florida, developed a banker plant system using papaya for the banker to rear the parasitoid wasp *Encarsia sophia* to protect tomato plants from whiteflies. Banker plants are additional plants grown with the primary crop to assist in establishing and maintaining biological control agents. Another system uses the predatory mite *Amblyseius swirskii* to protect plants from thrips using ornamental pepper banker plants. Mites fed a diet of ornamental pepper pollen alone develop at the same rate and have the same longevity as mites fed on chilli thrips. Mites are able to sustain themselves on the ornamental banker plants without pollen (flowers) or prey, which allows them to be introduced before pests arrive. This has application for use in seedling shipments as a preventative treatment during transport. The ornamental pepper and papaya banker plant systems have been used simultaneously in large-scale commercial poinsettia nurseries where pests were controlled. These two systems are being commercially produced by a private company due to the success of the pilot studies conducted on poinsettia.

Xiao, Y., Osborne, L., Chen, J., and McKenzie, C. 2013. Functional responses and prey-stage preferences of a predatory gall midge and two predacious mites with twospotted spider mites, *Tetranychus urticae*, as host. Journal of Insect Science 13:8.

Xiao, Y., Avery, P., Chen, J., McKenzie, C., and Osborne, L. 2012. Ornamental pepper as banker plants for establishment of *Amblyseius swirskii* (Acari: Phytoseiidae) for biological control of multiple pests in greenhouse vegetable production. Biological Control 63:279-286.

Xiao, Y., Chen, J., Cantliffe, D.l , McKenzie, C., Houben, K., and Osborne, L. 2011. Establishment of papaya banker plant system for parasitoid, *Encarsia sophia* (Hymenoptera: Aphilidae) against *Bemisia tabaci* (Hemiptera: Aleyrodidae) in greenhouse tomato production. Biological Control 58:239-247.

Xiao, Y., Osborne, L.S., Chen, J., McKenzie, C., Houben, K., and Irizarry, F. 2011. Evaluation of corn plant as potential banker plant for supporting predatory gall midge, *Feltiella acarisuga* (Diptera: Cecidomyiidae) against *Tetranychus urticae* (Acari: Tetranychidae) in greenhouse vegetable production. Crop Protection 30:1635-1642.

Genetic markers developed for rapid optimization of insect artificial diets. Insects are maintained in laboratory and production rearing systems globally to support research objectives and to produce beneficial insects that are competitive for deployment in biological control and in sterile insect release programs. Research designed to optimize insect diets requires iterative experiments in which diet formulations are created and tested by rearing insects through multiple life cycles to assess the quality and performance of the formulations. ARS scientists in Columbia, Missouri, utilized the effects of foods on gene expression to find new genetic markers that were then used to rapidly optimize insect diets. Strong gene responses that occur early in development are highly desired attributes of good biomarkers for diet formulation efforts. When applied to insects reared on artificial diets these studies revealed several new biomarkers; for example, low expression of a protein-modifying enzyme in eggs is a good marker of lipid deficiency, and high expression of an RNA binding protein in adults is a good marker for fertility. This approach has been adopted at the international level for use in diet optimization.

Zou, D.Y., Coudron, T.A., Liu, C., Zhang, L., Wang, M., and Chen, H. 2013. Nutrigenomics in *Arma chinensis*: transcriptome analysis of *Arma chinensis* fed on artificial diet and Chinese oak silk moth *Antheraea pernyi* pupae. PloS One 8(4):e60881.

Coudron, T.A., Chang, C.L., Goodman, C.L, and Stanley, D.W. 2011. Dietary wheat germ oil influences gene expression in larvae and eggs of the Oriental fruit fly. Archives Insect Biochemistry and Physiology 76:67-82.

How new biotypes of cereal aphids evolve and overcome aphid resistance genes in wheat. Since its introduction in 1986, the Russian wheat aphid has been the most destructive pest of wheat in the western United States. This pest was primarily managed by introgression of resistance genes into existing wheat varieties; however, biotypes of this aphid species have overcome this resistance in the field. For decades, biotype evolution was widely accepted to be a result of progressive mutations or new Russian wheat aphid biotype introductions into the United States because it was thought that the aphid reproduced solely by parthenogenesis. This belief was fueled by numerous unsuccessful attempts to find sexually reproducing aphids. To address the problem, ARS researchers in Stillwater, Oklahoma, in collaboration with scientists at Colorado State University, conducted studies on overwintering ecology and biotype distribution of the Russian wheat aphid in the western United States. The scientists found that biotypic variation in the Russian wheat aphid was extensive and that sexual reproduction occurred in high elevations bordering the Rocky Mountains. Screening the progeny from sexually

reproducing Russian wheat aphids has identified 40 new biotypes and identified this mode of reproduction as a major source of the aphid's biotypic diversity. Evaluations of these progeny have identified sources of resistance in wheat and barley that are resistant to all known biotypes in the United States and provided much needed direction in State and Federal cereal breeding programs that are developing Russian wheat aphid-resistant wheat varieties. Development of new Russian wheat aphid-resistant varieties will significantly reduce insecticide usage, which is currently the only means of controlling the aphid in wheat.

Puterka, G.J., Hammon, R.W., Burd, J.D., Peairs, F.B., Randolph, T.L., and Cooper, W.R. 2012. Cyclical parthenogenetic reproduction in the Russian wheat aphid (Hemiptera: Aphididae) in the United States: Sexual reproduction and its outcome on biotypic diversity. Journal of Economic Entomology 105:1057-1068.

Puterka, G.J., Hammon, R.W., Burd, J.D., Peairs, F.B., Randolph, T., and Cooper, W.R. 2010. Host associations and incidence of *Diuraphis* spp. in the Rocky Mountain region of the United States and pictorial key for their identification. Journal of Economic Entomology 103:1875-1885.

Randolph, T.L., Peairs, F. Weiland, A., Rudolph, J.B., and Puterka, G.J. 2009. Plant responses to seven Russian wheat aphid biotypes (Hemiptera: Aphididae) found in the United States. Journal of Economic Entomology 102:1954-1959.

Weiland, A.A., Peairs, F.B., Randolph, T L., Rudolph, J.B., Haley, S.D., and Puterka, G.J. 2008. Biotypic diversity in Colorado Russian wheat aphid (Homoptera) populations. Journal of Economic Entomology 101:569-574.

Decision support systems to efficiently manage key insect pests of wheat and sorghum. Greenbug is a severe pest of wheat and sorghum in the Southern Plains region, and the Russian wheat aphid and sorghum headworm are severe pests of wheat and sorghum, respectively. About 20 percent of the approximate 20 million wheat and sorghum acres in the Southern Plains receive insecticidal application annually, at a cost of $40 million to $50 million. ARS researchers in Stillwater, Oklahoma, in collaboration with researchers at several universities in the affected area, conducted regional studies on economic injury levels, insecticidal control methods, and field sampling methods. The data collected were used to develop easy-to-use, computer-based decision support systems for managing these pests. The research significantly improved knowledge of the impact and management of these pests in their respective crops that is being used by pest managers throughout the Southern Plains. A recent survey indicated that approximately 25 percent of growers are using these tools.

Royer, T.A., Elliott, N.C., Giles, K.L., and Kindler, S.D. 2011. Field efficacy of wintertime insecticide applications against greenbugs, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) on winter wheat (*Triticum aestivum* L.). Crop Protection 30:826-832.

Multispectral remote sensing for area-wide monitoring of insect pests in wheat. Monitoring greenbug and Russian wheat aphid in agricultural fields is time consuming and costly, and more efficient methods are needed. Multispectral remote sensing has emerged as a potential tool for monitoring crop stress caused by pests and diseases, but challenges exist. Remote sensing alone frequently cannot differentiate between crop stress-causing factors such as drought, insect infestation, disease, and nutrient deficiency that produce similar spectral patterns and result in misidentification. ARS scientists at Stillwater, Oklahoma, in collaboration with colleagues at

Oklahoma State University and Texas A&M University, used spatial patterns analysis of multispectral imagery of wheat fields infested with Russian wheat aphid and greenbug and multispectral remote sensing for area-wide monitoring of these insect pests. They found that wheat fields where the predomiant source of plant stress was caused by Russian wheat aphid could be differentiated with 97 percent accuracy from fields where other sources of plant stress, such as poor fertility and drought, predominated. The methodology relies on differentiating the cause of stress based on statistical analysis of shape, size, and other spatial attributes of stressed plants within a field. The system is cost effective because a large spatial area can be imaged quickly and at a cost of $0.25 per acre or less. Issues that need to be addressed to produce an operational monitoring system include automation of image download, spatial pattern analysis, and discriminant function modeling and identification of the most cost-effective and accurate data acquisition platform and system from among available options. Expanding the methodology to include a broader range of pests also would help create demand for monitoring based on the methodology.

Backoulou, G.F., Elliott, N.C., Giles, K. L., Catana, V., Phoofolo, M. W., Peairs, F.B., and Hein, G.L. 2010. Relationship between Russian wheat aphid population density and edaphic and topographic characteristics of wheat fields. Southwestern Entomologist 35:11-18.

Elliott, N.C., Mirik, M., Yang, Z., Jones, D., Phoofolo, M., Catana, V., Giles, K., and Michels Jr., G.J. 2009. Airborne remote sensing to detect greenbug stress to wheat. Southwestern Entomologist 34(3):205-211.

Mirik, M., Ansley, R.J., Michels Jr., G.J., and Elliott, N.C. 2009. Grain and vegetative biomass reduction by the Russian wheat aphid in winter wheat. Southwestern Entomologist 34(2):131-139.

Yang, Z., Rao, M.N., Elliott, N.C., Kindler, S.D., and Popham, T. 2009. Differentiating stress induced by greenbugs and Russian wheat aphids in wheat using remote sensing. Computers and Electronics in Agriculture 67(1-2):64-70.

New species of biological control agents against olive fruit fly discovered, released, and established. Discovered in California a decade ago, the olive fruit fly has become the most important olive pest and threatens the economic viability of the U.S. olive industry, which is valued at $40 million. In response, ARS scientists at the European Biological Control Laboratory in Montpellier, France, led explorations in Africa and Asia for effective natural enemies of the fly. Numerous agents were obtained and identified, and a number of these with the greatest potential were evaluated for efficacy and host specificity by cooperators in California (California Department of Food and Agriculture and the University of California). As a result, several parasitoid wasps were permitted by APHIS for field release in California. After several consecutive years of releases, recovery of one of these agents, the African parasitic wasp *Psyttalia lounsburyi* showed that it has established at several locations. A second, related species of *Psyttalia* from southern Africa also shows signs of establishment. Following unsuccessful efforts in Europe during past decades, this is the first successful establishment of an exotic natural enemy of olive fly from the fly's native range in Africa into other regions where olive culture has spread. Field release of a third species of *Psyttalia* from Pakistan is pending APHIS approval. In coordinated work, ARS scientists in Parlier, California, capitalizing on their partnership with the California Olive Committee, conducted laboratory, greenhouse, and field tests to determine olive fruit fly behavior and biology and, in collaboration with the APHIS-PPQ

and Moscamed Program in Guatemala, imported a parasitoid [*Psyttalia humilis* (Braconidae)], developed a rearing system, and released the biocontrol agent. Olive fruit fly was found to thrive in cool and humid coastal areas but not in hot and arid climates similar to the interior valleys where canning olives are grown. *P. humilis* was shown to have similar physical requirements and was released in many regions of the State. Resultant populations were readily recovered from the fruit fly host and became established in a coastal area of release. It is anticipated that a combination of *Psytallia* spp. parasitic wasps from the Montepellier and Parlier efforts will be needed to reduce olive fly populations to sustainable levels throughout California, especially in reservoir populations of wild and urban olives where fly management is not feasible.

Yokoyama, V.Y. 2012. Olive fruit fly (Diptera: Tephritidae) in California: Longevity, oviposition, and development in canning olives in the laboratory and greenhouse. Journal of Economic Entomology 105:186-195.

Yokoyama, V.Y., Wang, X.G., Aldana, A., Cáceres, C.E., Yokoyama-Hatch, H.A., Rendón, P.A., Johnson, M.W., and Daane, K.M. 2012. Performance of *Psyttalia humilis* (Hymenoptera: Braconidae) reared from irradiated host on olive fruit fly (Diptera: Tephritidae) in California. Environmental Entomology 41:497-507.

Cheyppe-Buchmann, S., Bon, M.C., Warot, S., Thaon, M., Jones, W., Fauvergue, X.,and Ris, N. 2011. Molecular characterization of the candidate biocontrol agent *Psyttalia lounsburyi* and its *Wolbachia* symbionts as a pre-requisite for future intraspecific hybridization. Biocontrol 56:713-724.

Hoelmer, K.A., Kirk, A.A., Pickett, C.H., Daane, K.M., and Johnson, M.W. 2011. Prospects for improving biological control of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), with introduced parasitoids (Hymenoptera). Biocontrol Science and Technology (21):1005-1025.

Yokoyama, V.Y., Cáceres, C.E., Kuenen, L.P.S., Wang, X.G., Rendón, P.A., Johnson, M.W., and Daane, K.M. 2010. Field perfomance and fitness of an olive fruit fly parasitoid, *Psyttalia humilis* (Hymenoptera: Braconidae) mass reared on irradiated Medfly. Biological Control 54:90-99.

Wang, X.-G., Nadel, H., Johnson, M.W., Daane, K.M., Hoelmer, K.A., Walton, V.M., Pickett, C.H., and Sime, K.R. 2009. Crop domestication relaxes both top-down and bottom-up effects on a specialist herbivore. Basic and Applied Ecology 10:216-227.

Bon, M.C., Jones, W., Hurard, C., Loiseau, A., Ris, N., Pickett, C., Estoup, A. and Fauvergue, X. 2008. Identification of 21 polymorphic microsatellites in the African parasitoid wasp, *Psyttalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae). Molecular Ecology Resources 8:930-932.

Mkize, N., Hoelmer, K., and Villet, M.H. 2008. A survey of fruit-feeding insects and their parasitoids occurring on wild olives, *Olea europaea* subsp. cuspidata, in the Eastern Cape of South Africa. Biocontrol Science and Technology 18:991-1004.

Breeding solid stem wheat for resistance to sawfly generally disrupts biological control. Annual regional losses due to wheat stem sawfly damage are estimated at more than $100 million a year. ARS researchers in Sidney, Montana, determined that increasing stem solidity is associated with an approximately four-fold reduction in average native parasitism rates of wheat stem sawfly. The researchers also determined that wheat genotype had a significant influence on levels of parasitism, independent of its effects on stem solidity. Overall, results suggest that

although increasing stem solidity generally reduces parasitism, significant genotypic variability in average parasitism levels already exists within solidity categories. Thus, it may be possible to select resistant solid-stemmed genotypes that also maintain relatively high parasitism levels. This study is among the first to demonstrate a strong direct effect of genotypic variation in stem solidity on parasitism of grass mining insects, with important applied implications.

Rand, T.A., Waters, D.K., Shanower, T.G., and Berzonsky, W.A. 2012. Effects of genotypic variation in stem solidity on parasitism of a grass-mining insect. Basic and Applied Ecology 13(3):250-259.

Managing the insect vector of zebra chip disease of potato. Zebra chip, a new and economically important disease of potato, has caused millions of dollars in losses to the potato industry in the United States, Mexico, Central America, and New Zealand. The disease is caused by the bacterium *Candidatus* Liberibacter solanacearum. ARS scientists in Wapato, Washington, determined that the bacterium was transmitted to potato by the potato psyllid, and they demonstrated that as few as one psyllid can effectively transmit the pathogen to a potato and cause zebra chip by feeding on a plant for as little as six hours. In addition, the scientists showed that it takes 3 weeks for zebra chip symptoms to develop in potato tubers after plant exposure to infective psyllids. The scientists, along with ARS colleagues in Prosser, Washington, and Weslaco, Texas, and university and industry researchers, demonstrated that several biorational insecticides, including mineral oils, entomopathogenic fungi, and kaolin, were effective in controlling the potato psyllid. The scientists also found that the timing of potato planting in some affected regions can significantly reduce zebra chip incidence by avoiding psyllid transmission of the bacterium. This zebra chip research and the development of effective management strategies for its insect vector earned the researchers the 2012 Integrated Pest Management Team Award by the Entomological Society of America.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Goolsby, J.A., Ochoa, A.P., Trevino, J., and Schuster, G. 2012. Impact of potato planting time on incidence of potato zebra chip disease in the Lower Rio Grande Valley of Texas. Southwestern Entomologist 37:253-262.

Buchman J.L., Heilman, B.E., and Munyaneza, J.E. 2011. Effects of *Bactericera cockerelli* (Hemiptera: Triozidae) density on zebra chip potato disease incidence, potato yield, and tuber processing quality. Journal of Economic Entomology 104:1783-1792.

Lacey, L.A., Liu, T.X., Buchman, J.L., Munyaneza, J.E., Goolsby, J.A., and Horton, D.R. 2011. Entomopathogenic fungi (Hypocreales) for control of potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) in an area endemic for zebra chip disease of potato. Biological Control 56:271-278.

Yang, X.B., Zhang, Y.M., Hua, L., Peng, L.N., Munyaneza, J.E., and Liu, T.X. 2010. Repellency of selected biorational insecticides to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae). Crop Protection 29:1320-1324.

Munyaneza, J.E., Crosslin, J.M., and Upton, J.E. 2007. Association of *Bactericera cockerelli* (Hemiptera: Psyllidae) with 'Zebra Chip', a new potato disease in southwestern United States and Mexico. Journal of Economic Entomology 100:656-663.

Area-wide control of fruit flies in Hawaii. Tephritid fruit flies are one of the most economically important invasive threats to the United States, and they are established in Hawaii, where their larvae spoil fruit and threaten export markets. For the mainland, APHIS maintains a Fruit Fly Detection and Exclusion Program. To counter the threat of fruit flies decimating Hawaii's export economy, ARS, APHIS, the University of Hawaii, and the Hawaii Department of Agriculture established the Hawaii Area-Wide Integrated Pest Management of Fruit Flies in Hawaiian Fruits and Vegetables Program in 2000. The goal was to develop and integrate biologically based pest technology into a comprehensive IPM package that was economically viable, environmentally friendly, and sustainable. Funded until 2009, the area-wide program included operational research, education, and assessment components. Technologies developed by ARS scientists and deployed by growers and partners included field sanitation, application of protein bait, male and female annihilation with male lures and other attractants, sterile insect releases, and conservation and release of beneficial parasitoids. Implementation of the program in many cases reduced the use of organophosphate insecticides by 75 to 90 percent, and nearly 1,000 farmers and homeowners have adopted the program. A cost-benefit analysis found that the program was easy to use by local farmers and residents and created as much as a 32 percent return on investment. The program has been nationally recognized with seven major awards and has received international attention. Technology transfer and training have been taken to Thailand, China Taiwan, French Polynesia, Guam, the Northern Marianas, Fiji, and various western African countries. Although funding has ended, the program is still being promoted by the University of Hawaii Extension Service within Hawaii through field days and distribution of an updated DVD. This more environmentally sound approach is facilitating development of systems approaches for movement of fruits and vegetables impacted by fruit fly quarantines. The 1-2-3 approach, a management strategy developed by ARS that encourages (1) field sanitation, (2) the use of monitoring traps to detect pest populations, and (3) the use of protein-based sprays is being adopted worldwide.

Biological control has been shown to be a fourth successful approach. For example, in Tahiti (French Polynesia), ARS scientists led the establishment of the parasitoid *Fopius arisanus*, which, with the 1-2-3 approach, reduced the Oriental fruit fly and two other fruit fly species (Queensland fruit fly and *Bactrocera kirki*) by approximately 90 percent and subsequently reduced economic damage to tree fruits. From a small laboratory, the French Polynesians have been able to rear and release the parasitoids against the fly, as the latter has spread throughout the Society Islands and to the Marquesas Islands, expanding the local program to an area-wide or regional program. Establishment of *F. arisanus* in French Polynesia is the most successful example of classical biological control of fruit flies in the Pacific outside of Hawaii and serves as a model for introductions elsewhere.

Furthermore, the wasp itself has been exported and released in Senegal in March 2013 to control *Bactrocera invadens* and to a quarantine facility in Sao Paulo, Brazil, in 2013 for eventual release in Northern Brazil against *Bactrocera carambolae*. These two collaborations and export of *F. arisanus* were made possible by methodology for shipping parasitoids in infested pupae developed by ARS scientists.

Fruit flies must be reared to, in turn, rear the wasps. This is an expensive undertaking due to the need to dispose of the huge waste stream produced. ARS scientists in Hilo, Hawaii developed a

liquid diet technology, which uses sponges floating in the liquid diet to provide a platforms for insects to grow while obtaining nutrients from the diet, as a solution for rearing tephritids. Since 2006, this technology has been transferred to more than 36 countries for evaluation on 25 species. This transfer includes invited on-site demonstrations in four of the largest Mediterranean fruit fly rearing facilities and transfer of starter kits to the other countries. Thirteen of the recipient institutions have successfully evaluated liquid diet rearing and have adopted it. The most recent technology transfer was in February 2013 to Taiwan, which adopted liquid diet rearing for fruit flies used in molecular biological research. In addition to the fruit fly rearing technology transfer, the latest methodology for rearing the fruit fly parasitoid *F. arisanus* has also been disseminated via a peer-reviewed video article in the Journal of Visualized Experiments.

Chang, C.L., Coudron, T.A., Goodman, C., and Stanley, D. 2012. Larval dietary wheat germ oil influences age-specific protein expression in adults of the Oriental fruit fly. Journal of Insect Physiology 58:690-698.

Vargas, R.I., Leblanc, L., Putoa, R., and Pinero, J.C. 2012. Population dynamics of three *Bactrocera* spp. fruit flies (Diptera: Tephritidae) and two introduced natural enemies, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), after an invasion by *Bactrocera dorsalis* (Hendel) in Tahiti. Biological Control 60:199-206.

Vargas, R.I., Leblanc, L., Harris, E.J., and Manoukis, N.C. 2012. Regional suppression of *Bactrocera* fruit flies (Diptera: Tephritidae) in the Pacific through biological control and prospects for future introductions into other areas of the world. Insects 3(3):727-742.

Nicholas, C.M., Scott, M.G., Seo, D.M., McKenney, M.P., Vargas, R.I., and Jang, E.B. 2011. An optimized protocol for rearing *Fopius arisanus*, a parasitoid of tephritid fruit flies. Journal of Visualized Experiments 53:e2901. http://dx.doi.org/10.3791/2901. [www.jove.com/video/2901/an-optimized-protocol-for-rearing-fopius-arisanus-parasitoid]

Vargas, R.I., Shelly, T.E., Leblanc, L., and Piñero, J.C. 2010. Recent advances in methyl eugenol and cue-lure technologies for fruit fly detection, monitoring and control. pp. 575-596, In: G. Litwack [editor], Vitamins and Hormones, Section: Pheromones, Vol. 83. Academic Press, Burlington.

Chang, C.L. 2009. Fruit fly liquid larval diet technology transfer and update. Journal of Applied Entomology 133:164-173.

Vargas, R.I., Mau, R.F.L., Jang, E.B., Faust, R.M., and Wong, L. 2008. The Hawaii Fruit Fly Area-Wide Pest Management Program. Pp. 300-325. In: O. Koul, O., Cuperus, G. W., and Elliott, N. C. (eds). Areawide IPM: Theory to Implementation. CABI Books, London.

Bacterial and fungal pathogens in stink bugs associated with boll rot of cotton. Stink bugs are polyphagous feeders on multiple crops and wild host plants. As they feed, they acquire opportunistic plant pathogens from environmental sources. Some of these pathogens are transmitted to cotton bolls, resulting in boll rot and subsequent yield losses. ARS scientists in College Station, Texas, identified the pathogens responsible for boll rot that are transmitted to cotton bolls by southern green stink bugs. These researchers determined that only two of the many pathogens found in the mouthparts, alimentary canal, or head of the stink bugs are actually transmitted into cotton bolls through bug feeding. This work provides an understanding of the role of stink bugs in transmitting pathogens to cotton bolls and will be used to develop pathogen-specific control measures for boll rot.

Esquivel, J.F. and Medrano, E.G. 2012. Localization of selected pathogens of cotton within the southern green stink bug. Entomologia Experimentalis et Applicata 142:114-120.

Medrano, E.G., Esquivel, J.F., Bell, A.A., Greene, J.K., Roberts, P.M., Bacheler, J.S., Marois, J.J., Wright, D.L., and Nichols, R.L. 2011. Analysis of microscopic cotton boll feeding injuries caused by southern green stink bug (Hemiptera: Pentatomidae). Southwestern Entomologist 36(3):233-245.

Esquivel, J.F., Medrano, E.G., and Bell, A.A. 2010. Southern green stink bugs (Hemiptera: Pentatomidae) as vectors of pathogens affecting cotton bolls - A brief review. Southwestern Entomologist 35:457-461.

Medrano, E.G., Esquivel, J.F., Bell, A.A., Greene, J., Roberts, P., Bachelor, J., Marois, J.J., Wright, D.L., Nichols, R.L., and Lopez, J. 2009. Potential for *Nezara virdula* (Hemiptera: Pentatomidae) to transmit bacterial and fungal pathogens into cotton bolls. Current Microbiology 59:405-412.

Mass production of biological control agents. Biological control methods are often not adopted for agriculture because mass production of natural enemies can be more costly than the use of pesticides. ARS scientists in Stoneville, Mississippi, are working to develop new technologies to mass produce biological control agents at commercially viable prices and encourage the growth of the biological control industry. The researchers have focused on improving the mass production of key predatory arthropods such as the predatory mite *Phytoseiulus persimilis*, the most important commercially available predator of the two-spotted spider mite. They developed new methods of producing this predator using continuous enclosed systems, thereby cutting costs of production by half. New rearing methods were developed for another important predator, the pink spotted lady beetle *Coleomegilla maculata*, and new inbred lines of this beetle were produced to enhance genomic research. These strains will be useful for *de novo* sequencing, dissecting biochemical pathways, and testing gene expression in stress conditions. New artificial diets have been developed for those pests and the mite *Amblyoseiulus swirskii* and the insidious flower bug (*Orius insidiosus*). New methods of encapsulating these artificial diets and novel methods of stimulating oviposition using artificial devices were developed under cooperative research agreements with industry partners. ARS research on the *in vivo* mass production of insect-killing (entomopathogenic) nematodes has yielded new technology to mass-produce the yellow mealworm *Tenebrio molitor*, including novel methods for obtaining eggs and separating larvae. Collectively, this research is building infrastructure for current and future biological control programs.

Morales-Ramos, J.A., Rojas, M.G., Shapiro-Ilan, D.I., and Tedders, L.W. 2013. Use of nutrient self-selection as a diet refining tool in *Tenebrio molitor* (Coleoptera: Tenebrionidae). Journal of Entomological Science 48:206-221

Allen, M.L. and Riddick, E.W. 2012. A system for harvesting eggs from the pink-spotted lady beetle. Psyche article no. 923653. DOI:10.1155/2012/923653

Morales-Ramos, J.A., Rojas, M.G., and Khan, D. 2012. System and Method for Production of Predatory Mites. U.S. Patent and Trademark Office, Patent no. 8,327,797 B1, December 2012.

Riddick, E.W. and Wu, Z. 2012. Mother – offspring relations: maternal size and prey quality affect egg size of an acariphagous lady beetle in culture. Psyche article no. 764350. DOI:10.1155/2012/764350.

Morales-Ramos, J.A., Rojas, M.G., Shapiro-Ilan, D.I., and Tedders, W.L. 2011. Automated Insect Separation System. U.S. Patent and Trademark Office, Patent no. 8,025,027 B1, September 2011.

Rojas, M.G. and Morales-Ramos, J.A. 2008. *Phytoseiulus persimilis* (Mesostigmata: Phytoseiidae) feeding on extrafloral nectar: Reproductive impact of sugar sources in presence of prey. Biopesticides International 4:1-5.

Area-wide control of European corn borer. Planting corn that is genetically engineered to make insect-killing proteins from *Bacillus thuringiensis* (Bt) suppresses populations of the European corn borer, a devastating pest of corn. In the United States, 67 percent of the corn is genetically engineered to express these insecticidal proteins. Farmers use Bt corn because of reduced crop damage, increased grain quality, and protected crop yields and a reduction in the need for chemical insecticides. ARS researchers in Ames, Iowa, and several Mid-western universities showed that area-wide pest suppression provides significant economic benefits to farmers that use Bt corn and even to neighboring farmers who grow non-transgenic varieties of corn. The researchers estimated that between 1996 and 2009, farmers in Iowa, Illinois, Minnesota, Nebraska, and Wisconsin received cumulative economic benefits of nearly $7 billion, with benefits of more than $4 billion for non-Bt corn farmers, due to both pest suppression and yield protection. Success of this collaboration resulted in the Integrated Pest Management Team Award from The Entomological Foundation in 2011.

Coates, B.S., Alves A., Walden, K., French, B.W., Miller, N.J., Abel, C.A., Sappington, T.W., Robertson, H.M., and Siegfried, B.D. 2012. Distribution of genes and repetitive elements in the *Diabrotica virgifera* genome estimated using BAC sequencing. Journal of Biomedicine and Biotechnology Article ID 604076. http://dx.doi.org/10.1155/2012/604076.

Hutchison, W.D., Burkness, E.C., Mitchell, P.D., Moon, R.D., Leslie, T.W., Fleischer, S.J., Abrahamson, M., Hamilton, K. L., Steffey, K.L., Gray, M. E., Hellmich, R.L., Kaster, L.V., Hunt, T.E., Wright, R.J., Pecinovsky, K., Rabaey, T.L., Flood, B.R., and Raun, E.S. 2010. Area-wide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330:222-225.

Coates, B.S., Sumerford, D.V., Kim, K.S., Miller, N.J., Sappington, T.W., Siegfried, B.D., and Lewis, L.C. 2009. Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. Journal of Heredity 100:556–564.

Management of Bt crops. Adoption of transgenic crops has not been universally supported out of concern for potential environmental effects and development of resistance to Bt toxins. Insect resistance to the Bt toxins, potential impact of Bt traits on non-target arthropods, and containment of Bt genes in the transgenic crop are among the concerns. ARS scientists in Maricopa, Arizona, and collaborators, working primarily with Bt cotton, have addressed a number of these concerns. These scientists demonstrated no adverse effects of ingestion of caterpillars fed on Bt crops by common predators such as ladybeetles, green lacewings, big-eyed bugs, and minute pirate bugs. The results support the contention that consumption of Bt-fed pests is not likely to adversely affect complexes of natural enemies needed for biological

control. Extensive meta-analyses of the world literature on Bt crops showed that effects on non-target arthropods were generally neutral and much less disruptive than alternative insecticides used to manage the Bt-targeted pest. Meta-analyses also validated the tier-testing system used by many regulatory authorities such as the EPA for assessing non-target impacts. Studies of movement of transgenic pollen by honey bees from seed alfalfa indicated generally low rates of gene flow between transgenic and conventional fields, with gene flow being undetectable at a distance of 5 miles from the source field. The information provided valuable guidelines to producers and seed companies regarding the degree of isolation necessary to eliminate unwanted movement of Bt genes in pollen. A large-scale, multi-State experiment showed that mandated refuges of non-Bt cotton were not necessary to avoid Bt resistance in the pink bollworm when sterile pink bollworm moths were released into the field as part of an eradication program. In response, refuge requirements by the EPA were relaxed, and yield losses associated with previously required non-Bt cotton have been reduced. These collective findings have been widely adopted by growers, industry, and regulatory agencies, and Bt crops continue to contribute to economically viable and environmentally safe systems of crop production.

Tian, J.C., Wang, X.P., Long, L.P., Romeis, J., Naranjo, S.E., Hellmich, R.L., Wang, P., Earle, E.D., and Shelton, A.M. 2013. Bt crops expressing Cry1Ac, Cry2Ab and Cry1F do not harm the green lacewing, *Chrysoperla rufilabris*. PLoS One 8(3):e60125.

Carrière, Y., Ellers-Kirk, C., Hartfield, K., Larocque, G., DeGain, B., Dutilleul, P., Dennehy, T.J., Marsh, S.E., Crowder, D.W., Li, X., Ellsworth, P.C., Naranjo, S.E., Palumbo, J.C., Fournier, A., Antilla, L., and Tabashnik, B.E. 2012. Large-scale, spatially explicit test of the refuge strategy for delaying insecticide resistance. Proceedings of the National Academy of Sciences of the United States 109(3):775-780.

Tian, J.C., Collins, H.L., Romeis, J., Naranjo, S.E., Helmich, R.L., and Shelton, A.M. 2012. Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one if its major predators. Transgenic Research 21:1303-1310.

Hagler, J.R., Mueller, S., Teuber, L.R., Machtley, S.A., and Van Deynze, A. 2011. Foraging range of honey bees, *Apis mellifera*, in alfalfa seed production fields. Journal of Insect Science 11:144.

Naranjo, S.E. 2011. Impacts of Bt transgenic cotton on integrated pest management. Journal of Agricultural and Food Chemistry 59:5842-5851.

Naranjo, S.E. and Ellsworth, P.C. 2010. Fourteen years of Bt cotton advances IPM in Arizona. Southwestern Entomologist 35:437-444.

Managing insecticide-resistant tarnished plant bug. Cotton farmers in the Mississippi Delta currently spend $75 per acre or more for control of the tarnished plant bug. This cost is about three times the cost of technology fees for Bt-cotton and about the same as total insecticide costs were prior to the availability of Bt-cotton. Cotton yields have dramatically increased during this period, offsetting the economic costs of insect control, but increased levels of insecticide resistance in tarnished plant bug threatens sustainable management systems and limits broad ecological stability opportunities. ARS scientists in Stoneville, Mississippi, in collaboration with scientists at several southern universities, have developed resistance monitoring tools to measure levels of tarnished plant bug insecticide resistance to pyrethroid,

organophosphorous, and neonicotinoid insecticides. The scientists have also associated critical resistance levels in the assay procedures with expected field control problems. This real-time distribution of information allows extension specialists and agricultural consultants to change insecticide chemistries based on regional monitoring results. Annual evaluations of the resistance monitoring trends are used to annually revise cotton insect control recommendations throughout the southern United States. This adaptability to a dynamic problem has helped farmers deal with a difficult pest problem and sustain cotton production in the region. Molecular and toxicological studies based on insects from problem fields have provided insight into resistance mechanisms and insect fitness costs associated with resistance genes. Demonstration of elevated P450 gene expression and increased tolerance to neonicotinoid insecticides are concerns for cross-resistance among the different classes of insecticide used for tarnished plant bug control. Populations selected in the laboratory have down-regulated reproductive- and digestive-related genes. The resistance detection tools developed by ARS scientists are allowing farmers to cope with resistant tarnished plant bug and maintain production through strategic use of traditional and new insecticides. Longer term solutions are being sought through an improved understanding of resistance mechanisms and alternatives to chemical control.

Allen, K.C., Jackson, R.E., Snodgrass, G.L., and Musser, F.R. 2012. Comparative susceptibilities of different life stages of the tarnished plant bug (Hemiptera: Miridae) to three classes of insecticides. Southwestern Entomologist 37:271-280.

Zhu, Y.C. and Luttrell, R. 2012. Variation of acephate susceptibility and correlation with esterase and glutathione S-transferase activities in field populations of the tarnished plant bug, *Lygus lineolaris.* Pesticide Biochemistry and Physiology 103:202-209.

Zhu, Y.C., West, S., Snodgrass, G., and Luttrell, R. 2011. Variability in resistance-related enzyme activities in field populations of the tarnished plant bug, *Lygus lineolaris*. Pesticide Biochemistry and Physiology 99:265-273.

Snodgrass, G.L., Abel, C., Jackson, R., and Gore, J. 2008. A bioassay for determining resistance levels in tarnished plant bug populations to neonicotinoid insecticides. Southwestern Entomologist 33:173-180.

Zhu, Y.C., Snodgrass, G.L., and Chen, M.S. 2007. Comparative study on glutathione S-transferase activity, cDNA, and gene expression between malathion susceptible and resistant strains of the tarnished plant bug, *Lygus lineolaris*. Pesticide Biochemistry and Physiology 87(1):62-72.

Benchmarks for insect resistance to Bt-cotton and Bt-corn. Commercial deployment of transgenic crops expressing insecticidal toxins from Bt requires EPA-approved resistance management plans and annual monitoring of targeted pest populations for changes in susceptibility. A bioassay procedure developed by ARS scientists 50 years ago to quantify potency of different Bt isolates has been universally adopted to compare susceptibilities of different lepidopteran pests to insecticidal proteins expressed in Bt crops. ARS scientists in Stoneville, Mississippi, in collaboration with researchers at the University of Arkansas and Mississippi State University, annually collect field populations of corn earworm and tobacco budworm and expose their progeny to Bt toxins in the diet-feeding assay. Comparison of observed survival rates to those recorded in benchmark studies have been used to confirm or refute suspected field control problems. The diet-feeding assays are not perfect because of a

number of experimental problems associated with rearing field colonies of insects and sampling inefficiencies, but the procedure remains a critical link to benchmark studies. Resulting data have been widely used in policy and strategic discussions of Bt resistance management. This annual process of measuring susceptibilities of field populations of corn earworm and tobacco budworm is an important aspect of public-sector stewardship of Bt crops in the southern United States.

Luttrell, R.G. and Jackson, R.E.. 2012. *Helicoverpa zea* and Bt cotton in the United States. GM Crops and Food Biotechnology in Agriculture and the Food Chain 3(3):213-227.

Blanco, C.A., Andow, D., Gould, F., Abel, C.A., Sumerford, D.V., Hernandez, G., Lopez, J., Adams, L., Groot, A., Leonard, R., Parker, R., Payne, G., Perera, O.P., and Teran-Vargas, A. 2009. *Bacillus thuringiensis* Cry1Ac resistance frequency in tobacco budworm (Lepidoptera: Noctuidae). Journal of Economic Entomology 102:381-387.

Luttrell, R.G. and Ali, M I. 2009. Variability in the response of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) to Cry1Ac and Cry2Ab2 in diet incorporation assays. Resistance Pest Management Newsletter 19:33-36.

Blanco, C.A., Storer, N.P., Abel, C., Jackson, R., Leonard, R., Lopez, J.D., Payne, G., Siegfried, B.D., Spencer, T., and Terán-Vargas, A.P. 2008. Baseline susceptibility of tobacco budworm (Lepidoptera: Noctuidae) to Cry1F toxin from *Bacillus thuringiensis*. Journal of Economic Entomology 101:168-173.

Federal Section 18 Crisis Exemptions. To combat quickly invading new pests for which effective chemical controls are lacking, ARS has identified key insecticides for their management and collaborated with affected States to appeal to EPA for Section 18 Crisis Exemptions for their use. This is a temporary exemption from labeling requirements that allows use of a pesticide to protect a highly threatened crop.

Imidicloprid to control West Indian canefly. Sugarcane in Louisiana is attacked by a number of insects representing several important groups (beetles, moth stem borers, sap-feeders). In 2012, a new pest problem occurred when unusually high numbers of West Indian canefly (*Saccharosyndne saccharivora*) were encountered infesting much of the sugarcane grown in Louisiana. In response to grower concerns, ARS scientists in Houma, Louisiana, experimentally identified effective and safe insecticides for control of the canefly, as well as determined yield losses ascribed to canefly infestations. Based on ARS research, the Louisiana Department of Agriculture and Forestry was successful in obtaining a Federal Section 18 Crisis Exemption for the insecticide imidacloprid. Approximately 16,000 acres of sugarcane was treated over the 15 days that the Crisis Exemption was valid. ARS research results showed that canefly feeding resulted in an approximately 23 percent reduction in sugar yield. An economic analysis conducted by the Louisiana State University AgCenter showed a gain of $397 per acre resulting from insecticide treatment, with a total economic gain for the 16,000 acres treated at $6.5 million. These data, plus additional data generated by cooperators, were used to support a request for a Section 18 Specific Exemption for imidacloprid use for canefly control in 2013.

Pesticides to control brown marmorated stink bug. The brown marmorated stink bug, *Halyomorpha halys* (Stål), an invasive species from Asia, caused devastating losses to tree fruit in the mid-Atlantic states in 2010. The U.S. Apple Association estimated that the brown marmorated stink bug feeding resulted in losses of $37 million to apple growers alone. Furthermore, there were no specific recommendations available for management of this invasive species. In response, ARS scientists, along with university partners, rapidly developed bioassay techniques for evaluating insecticide efficacy in early 2011. Using these techniques, the team rapidly generated pesticide efficacy data over several months that was used by cooperative extension across the mid-Atlantic to generate specific management recommendations for the 2011 season and beyond. These data also served as the basis for a successful Federal Section 18 Crisis Exemption application for dinotefuran usage in peaches and apples in seven States from 2011to 2013. During that time, these data also led to a second successful Section 18 exemption for bifenthrin usage.

Lee, D-H., Wright, S.E., and Leskey, T.C. 2013. Impact of insecticide residue exposure on the invasive pest, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae): analysis of adult mobility. Journal of Economic Entomology 106(1):150-158.

Leskey, T.C. Short, B.D, and Lee, D-H. 2013. Efficacy of insecticide residues on adult *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. Pest Management Science DOI:10.1002/ps.3653 (accepted).

White, B., Dalley, C., and Viator, B. 2013. West Indian canefly (Fulgorid) experiences from the 2012 season. Sugar Bulletin 91:21-23.

Leskey, T.C., Lee, D-H. , Short, B.D. and Wright, S.E. 2012. Impact of insecticides on the invasive *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae): analysis on the insecticide lethality. Journal of Economic Entomology 105:1726-1735.

IR-4 Projects. Growers of specialty (minor acreage) crops such as fruits, vegetables, mint, hops, herbs, and spices generally lack the type of effective pesticides that are available to growers of major crops. Minor crops are grown on less than 300,000 acres per crop as compared to the 12 to 72 million acres per major crop, and thus there is no economic incentive for pesticide manufacturers to develop the labeling data for these minor acreage crops. ARS participates in a State-Federal program known as Interregional Research-4 (IR-4) to assist in the development of data to support pesticide residue tolerances that are established by the U.S. Environmental Protection Agency and used by the pesticide registrants to add the crops to their labels. ARS collects information that is used by pesticide registrants to add these specialty crops to their use labels. This work responds to three commodity needs: residue analysis for specialty food crops to ensure safety of treated food, determination of phytotoxicity for nursery and floral crops, and, providing crop grouping evidence. The current EPA crop grouping approach allows data developed on a few representative crops to suffice for many crops, thereby increasing the efficiency of obtaining EPA tolerances. The IR-4 program annual reports provide more information on the pesticides tested and the impact of the program at this link: http://ir4.rutgers.edu/Other/annreports.html.

Data to support the registration of pesticides for minor use and specialty food crops. From 2007 to 2012, ARS researchers at Charleston, South Carolina; Maricopa, Arizona; Salinas, California; Tifton, Georgia; Prosser, Washington; Wapato, Washington; Weslaco, Texas; and Wooster, Ohio, established 596 pesticide/food crop combinations in the field to treat with pesticides and ARS researchers at Beltsville, Maryland; Tifton, Georgia; and Wapato, Washington, analyzed 762 pesticide/crop combinations in the laboratory for pesticide residues on treated crops. ARS contributed data for tolerances on 166 crops and 94 pesticides to be used by registrants for labeling their products for these uses that would make them available to specialty crop growers.

Pesticides for growers of nursery and floral crops. The IR-4 Ornamental Horticulture Program supports an industry valued at over $11.7 billion in annual sales with crops that are grown under a number of conditions such as nurseries, greenhouses, and tree farms. The plants can be in beds, containers, or in-ground. The growers are involved in a number of diverse markets, including flowers, bulbs, house plants, perennials, trees, shrubs, and nonbearing fruit trees. The primary objective of the IR-4 program for nursery and floral crops is to generate data on crop phytotoxicity. These plants have a very high value per acre, and therefore the potential for large losses in the case of crop failure, which can be a major deterrent to pesticide registrants labeling their products for these uses. The enormous number of plants and varieties present a challenge for crop safety when treated with pesticides, so considerable effort must be spent in developing phytotoxicity data so that pesticide manufacturers will add these crops to their labels. From 2007 to 2012, ARS researchers in Charleston, South Carolina; Corvallis, Oregon; Prosser, Washington; Tifton, Georgia; Wapato, Washington; and Wooster, Ohio, and University of Maryland cooperators, supported by ARS funds, established 1,315 pesticide/crop combinations in the field to treat ornamental plants with pesticides and evaluate them for crop safety. A select number of these combinations were also evaluated to see how well the pesticide performed against the target pest. For 2007–2012, ARS contributed data toward the registration of uses for 256 crops and 73 pesticides that are now available to growers of florist and nursery crops to reduce losses from pests.

Expanding EPA crop-grouping scheme to assist pest control for minor crops. Crops are grouped according to their taxonomic classification as well as other factors, including geographical distribution, cultural practices, pest problems, and residue levels on edible portions of the crops. Crop grouping adds about four crops for each crop tolerance obtained by IR-4, which results in a savings of about $450,000 per tolerance. An ARS scientist serves as the USDA member of the EPA/Office of Pesticide Programs Rule Making Workgroup for the Crop Grouping Regulation and provided advice and peer review of crop grouping technical documents developed by the Crop Grouping Coordinator of EPA. From 2007 to 2012, the following crop groups were reviewed, and rules were published in the Federal Register: Bulb vegetables group, 3; Fruiting vegetables group, 8; Citrus group, 10; Pome fruit group, 11; Stone fruit group, 12; Berries and small fruit group, 13; Tree nuts group, 14; Oilseeds group, 20; and Edible fungi group, 21. The seven revised groups and two new groups resulted in the addition of 239

commodities to the crop grouping scheme. An ARS scientist also serves on the IR-4 International Crop Grouping Consulting Committee that provides input to IR-4 in the determination of the crop-grouping scheme proposed to EPA and that subsequently results in a regulation for each crop group proposed.

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COMPONENT III: Protection of Natural Ecosystems

Invasive pests are found in all major habitats across the country. In response to the problem of invasive species, Executive Order 13112, issued in 1999, established the National Invasive Species Council, with the goal of minimizing harm to the economy, the environment, and human health. In the last 5 years, NP 304 scientists have responded by focusing on invasive insects and weeds found in urban landscapes, forests, and wetlands. The scientists have focused on early detection and rapid response and, when possible, developed biological and cultural methods to control these pests.

Research for Component 3 resulted in a number of accomplishments that improved entire ecosystems. Successful biological control of the invasive paperbark tree in the Florida Everglades saved that habitat for native plants and wildlife. A bi-national effort with Mexico reduced the threat of cactus moth through the sterile insect technique, protecting the unique cactus flora of two countries and protecting agricultural cactus production. ARS worked with the Canadian Forest Service to develop an emerald ash borer attractant, a key tool for this pest that threatens shade trees and forests. Also, ARS developed biological control of the giant reed (*Arundo donax*) in the Rio Grande valley, where it threatens water supplies and border security. These representative accomplishments show the strong impact of these programs and reflect integrated teamwork between ARS Overseas Biological Control Laboratories, ARS domestic laboratories, academic partners, and foreign governments.

Identifying natural enemies of exotic invasive pests requires research contributions from the ARS Foreign Laboratories in Argentina, Australia, China, France, and Greece. Research was conducted in collaboration with university and industry scientists, Mexican scientists and regulators; and research outputs are being used by extension specialists, Federal, State, university, industry, and international researchers, and U.S. and international regulators.

The NP 304 Action Plan included two Problem Statements that were expected to guide the 5-year research plan and the development of the anticipated products in this Component. The Problem Statements and the research accomplishments that address each of them are presented below.

PROBLEM STATEMENT IIIA: *Insects*

With the expansion of world trade, accidental introductions of exotic insects pose an increasing threat to our natural ecosystems. In many instances, pesticides are not a viable option because of statutory restrictions, potential adverse effects on non-target organisms, and/or the large acreages requiring treatment. Therefore, safe, effective, biologically based management strategies and controls need to be developed to mitigate the threats of these pest insects. The importance of this area of research is likely to increase as climate change disrupts ecosystems, creating large areas of disturbed habitats that are more susceptible to colonizing invasive species.

Research under this Problem Statement focused on controlling invasive pest insects—particularly the emerald ash borer, Asian long-horned beetle, and cactus moth—in urban landscapes, forests, and wetlands. These are high-profile pests that could dramatically alter forests and city landscapes, impacting natural habitats and the lumber and nursery industries and potentially costing billions of dollars in lost revenue and control treatment. In this 5-year period, ARS scientists made progress toward emerald ash borer control by identifying and commercializing a pheromone attractant and establishing an exotic natural enemy (parasitoid wasp). The scientists also developed tools for eradicating the Asian long-horned beetle, as well as new sterile insect techniques used to eradicate the cactus moth from Mexico.

Successful development of classical biological control to manage the emerald ash borer. The emerald ash borer is a devastating invasive pest that has killed tens of millions of North American ash trees (*Fraxinus* spp.) across the United States since its introduction in 2002. Regulatory efforts have not contained the borer's spread, and chemical control over huge acreages is both prohibitively costly and harmful to the environment. Classical biological control is, nevertheless, a promising approach. Working with the U.S. Forest Service and APHIS, ARS researchers in Newark, Delaware, introduced three natural enemies (parasitoid wasps) of the emerald ash borer in 14 of 18 States with infestations. Recent field studies conducted in Michigan, the epicenter of the borer infestation, showed that one of the introduced parasitic wasps, *Tetrastichus planipennisi*, has become established and widespread, with increased populations 4 years after its initial field release in 2008. This released wasp, together with native natural borer enemies (woodpeckers, parasitic wasps, and pathogens), have contributed to a 75-percent reduction in the pest's larvae. In addition, ARS has also successfully developed an alternative host plant-based method for rearing emerald ash borer parasitoids. This method has now been adopted by APHIS to mass-produce introduced parasitoids for field releases. Most recently, a new parasitoid wasp, *Spathius galinae*, has been discovered in Russia and imported to the ARS quarantine facility in Newark, Delaware, for potential introduction against emerald ash borer on large ash trees.

Duan, J.J., Bauer, L.S., Abell, K.J., Van Driesche, R.G., and Lelito, J.P. 2013. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: Potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 106(3):1145-54.

Duan, J.J. and Oppel, C.B. 2012. Critical rearing parameters of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) as affected by host-plant substrate and host-parasitoid group structure. Journal of Economic Entomology 105:792-801.

Duan, J.J., Yurchenko, G., and Fuester, R.W. 2012. Occurrence of emerald ash borer (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far East. Environmental Entomology 41:245-254.

New chemical attractants for the detection, monitoring, and control of the emerald ash borer. Sex pheromones (chemicals produced by insects to attract mates) are needed in pest management programs to detect, track, and monitor insect populations. ARS scientists in Peoria, Illinois, were the first to identify the emerald ash borer sex pheromone. In collaboration with scientists at APHIS and the Canadian Forest Service, ARS researchers showed that the sex pheromone can be mixed with a chemical produced by ash trees to make a highly effective attractant for adult emerald ash borers. According to the Canadian collaborators, who are synthesizing the

pheromone, this newly developed attractant mixture is less costly to produce than the ash tree oils currently being used for emerald ash borer monitoring and surveillance. Lures containing this attractant have been commercialized by an industry partner. In addition, the research team found attractants for the parasitic wasp *Spathius agrili*, a natural enemy of the emerald ash borer and a potential biocontrol agent. The attractants are being used by APHIS scientists to track *S. agrili* to monitor its effectiveness for controlling emerald ash borer infestations

Cossé, A.A., Petroski, R.J., Zilkowski, B.W., Vermillion, K., Lelito, J.P., Cooperband, M .F., and Gould, J.R. 2012. Male-produced pheromone of *Spathius agrili*, a parasitoid introduced for the biological control of the invasive emerald ash borer, *Agrilus planipennis*. Journal of Chemical Ecology 38:389-399.

Crook, D.J., Khrimian, A., Cossé, A.A., Ivich, F., and Mastro, V.C. 2012. Influence of trap color and host volatiles on capture of the emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 105:429-437.

Ryall, K.L., Silk, P.J., Mayo, P., Crook, D., Khrimian, A., Cossé, A.A., Sweeney, J., and Scarr, T. 2012. Attraction of *Agrilus planipennis* (Coleoptera: Buprestidae) to a volatile pheromone: Effects of release rate, host volatile, and trap placement. Environmental Entomology 41:648-656.

Silk, P.J., Ryall, K., Mayo, P., Lemay, M.A., Grant, G., Crook, D., Cossé, A.A., Fraser, I., Sweeney, J.D., Lyons, D.B., Pitt, D., Scarr, T., and MaGee, D. 2011. Evidence for a volatile pheromone in *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) that increases attraction to a host foliar volatile. Environmental Entomology 40:904-916.

Bartelt, R.J., Cossé, A.A., Zilkowski, B.W., and Fraser, I. 2007. Antennally active macrolide from the emerald ash borer *Agrilus planipennis* emitted predominantly by females. Journal of Chemical Ecology 33:1299-1302.

Improved detection and cultural management of emerald ash borer infestations. Knowledge of colonization patterns of the emerald ash borer will allow development of efficient management tactics for this invasive pest. ARS researchers in Ithaca, New York, in cooperation with scientists from the State University of New York, determined the extent of an emerald ash borer infestation in western New York by using more than 100 individual (sentinel) girdled ash trees located up to 10 kilometers away from the center of the infestation. Clusters of girdled ash trees were established near the center to act as sinks to attract the beetles. The researchers found that trees within the clusters were more heavily infested (nine larvae per square meter of bark) than were sentinel trees or ungirdled trees. This indicates that clusters of girdled trap trees can help lower local densities of the borer, a finding now being used by land managers in New York State. In a related study, the researchers modeled beetle distribution based on sampling 1-meter increments of 71 ash trees. The probability of detecting the emerald ash borer was highest at tree diameters of 19 centimeters and at heights of 14 meters. The researchers found that the degree of bark roughness was not a useful predictor of beetle presence. Results of these studies are being used by forest managers in New York State to improve emerald ash borer detection and management.

Fierke, M.K., Whitmore, M.C., Foelker, C., Vandenberg, J.D., and Carlson, J. 2013. Delimitation and management of emerald ash borer (Coleoptera: Buprestidae): case study at an outlier infestation in southwestern New York State, United States of America. The Canadian Entomologist 145(5):577-587. DOI:10.4039/tce.2013.39.

Foelker, C.J., Vandenberg, J.D., Whitmore, M., and Fierke, M.K. 2013. Modeling *Agrilus planipennis* (Coleoptera: Buprestidae) within-tree colonization patterns and development of a subsampling technique. Environmental Entomology 42(3):532-538. DOI:10.1603/EN12276.

New tools for early detection of Asian longhorned beetle. The Asian longhorned beetle is an invasive insect pest of hardwood trees (particularly maples) and a $670 billion threat to the U.S. economy. Since 1996, APHIS has spent more than $520 million to eradicate the beetle, including the removal of more than 70,000 infested shade trees. Further understanding of the beetle's biology and behavior is necessary to improve survey and detection, the single most costly and important component for successful eradication. In support of that effort, ARS scientists in Newark, Delaware, have developed modeling software that predicts when the beetles are most likely to be out of the trees (and thus most susceptible to eradication) and reference material illustrating visible signs of damage on hardwood trees that helps park rangers and homeowners spot infestations.

For the modeling software, the researchers related beetle emergence to ambient temperature, leading to a degree-day model for predicting when adult beetles emerge from infested trees and attack additional trees. The scientists showed that the rate and distance at which adult beetles disperse from infested trees is regulated by the density of beetles within infested trees and the density of preferred tree species in infested areas, leading to a dispersal model for predicting where adult beetles and infested trees most likely occur. Based on ARS research, APHIS incorporated degree-day model predictions into its Plant Pest Forecasting System (NAPPFAST) to produce geo-referenced risk assessments of beetle emergence across the United States. Furthermore, from 2008 to present, the ARS scientists have calculated and distributed updated weekly predictions of adult beetle emergence for over 100 targeted high-risk locations across the United States, Canada, and Europe. Through multiple national and international collaborations, many organizations and agencies worldwide have used these dispersal model predictions to identify and delineate high-risk areas (boundaries) and thereby determine where to focus survey efforts to detect infested trees. Equally important, APHIS uses these dispersal model predictions to design, produce, and establish precision geo-referenced networks (a grid) of locations, including in Worcester, Massachusetts, and Bethel, Ohio, where pheromone traps are deployed for detection of the adult beetles in infestations. Predictions from these models currently serve APHIS and State and local agencies as the basis for determining when to implement and where to focus survey, treatments, and other adaptive management strategies.

The ARS researchers' evaluation of the visible symptoms of beetle damage led to the determination that the tree attacked by the beetle varies by tree species and by the position and age (number of years) of the symptoms. A compilation of these symptoms was used to develop the first and most widely used training guide for identification and detection of infested trees, which was adopted for training inspectors and used in educational outreach to the public. ARS produced the visual reference material in collaboration with the Canadian Forest Service and Canadian Food Inspection Service (www.glfc.forestry.ca/VLF/invasives/alhbdetecguide_e.pdf). More than 5,000 copies have been distributed in Canada, Europe, Australia, New Zealand, and across Asia, where they have significantly improved survey efficiency.

These science-based tools have reduced the cost and improved the effectiveness of early detection of existing beetle infestations. Collectively, implementation of these tools has resulted in the successful eradication of the Asian longhorned beetle in Chicago, Illinois (2008), Islip (2011) and Manhattan, New York (2013), New Jersey (2013), and Toronto, Canada (2013). Programs are ongoing in New York State, Massachusetts, and Ohio.

Smith, M.T., Turgeon, J.J., de Groot, P., and Gasman, B. 2009. Asian Longhorned beetle *Anoplophora glabripennis* (Motschulsky): Lessons learned and opportunities to improve the process of eradication and management. American Entomologist 55(1):21-25.

Turgeon, J.J., Pedlar, J., de Groot, P., Smith, M.T., Jones, C., Orr, M., and Gasman, B. 2009. Density and location of simulated signs of injury affect efficacy of ground surveys for Asian Longhorned beetle. Canadian Entomologist 142:80–96.

Ric, J., de Groot, P., Gasman, B., Orr, M., Doyle, J., Smith, M.T., Dumouchel, L., Scarr, T., and Turgeon, J.J. 2007. Detecting signs and symptoms of Asian Longhorned beetle injury: A training guide. Natural Resources Canada, Canadian Forest Service 131pp.

Advanced polydnavirus-based strategies for disruption of caterpillar pest immune systems. Parasitoid wasp polydnaviruses useful for biocontrol of lepidopteran pest insects have not previously been characterized at any molecular genetic level. ARS scientists in Beltsville, Maryland, discovered how pro-viral and infectious forms of polydnavirus assist wasp survival, which may lead to new biocontrol strategies based on disruption of insect pest immune systems. The scientists determined the structure and organization of the *Glyptapanteles (*braconid) polydnavirus provirus as multiple loci containing one or many viral segments, flanked and separated by wasp gene-encoding DNA, and the mechanisms by which the production of virion segments occur prior to virus packaging. They determined that a polydnavirus sugar transporter gene family originating from the wasp chromosome was packaged in virions for delivery as virulence factors to the host gypsy moth. This research has enabled analyses of chromosomal homologies among polydnaviruses in other systems to understand and exploit polydnavirus as regulators in the antagonistic relationship between parasitoids and their hosts.

Gundersen-Rindal, D.E. and Pedroni, M.J. 2010. Larval stage Lymantria dispar microRNAs differentially expressed in response to parasitisation by *Glyptapanteles flavicoxis* parasitoid. Archives Virology 155:783-787.

Desjardins, C.A., Gundersen-Rindal, D.E., Hostetler, J.B., Tallon, L.J., Fadrosh, D.W., Fuester, R.W., Pedroni, M.J., Haas, B.J., Schatz, M.C., Jones, K.M., Crabtree, J., Forberger, H., and Nene, V. 2008. Comparative genomics of mutualistic viruses of *Glyptapanteles* parasitic wasps. Genome Biology 9(12):R183.

Desjardins, C.A., Gundersen-Rindal, D.E., Hostetler, J.B., Tallon, L.J., Fuester, R.W., Schatz, M.C., Pedroni, M.J., Fadrosh, D.W., Haas, B.J., Toms, B.S., Chen, D., and Nene, V. 2007. Structure and Evolution of a Proviral Locus of *Glyptapanteles indiensis* Bracovirus. BMC Microbiology 7:61.

Development of area-wide management technology against the invasive cactus moth. The cactus moth *(Cactoblastis cactorum),* native to Argentina, was found in Florida in 1989 and now threatens cactus-based agriculture and desert ecosystems in the southwestern United States and Mexico. ARS scientists in Gainesville (and a Tallahassee worksite), Florida, collaborated with regulatory agencies in the United States and Mexico to develop and deploy monitoring

technology and an area-wide management program against the moth. Traps baited with an ARS-developed synthetic female cactus moth pheromone were adapted by State and Federal agricultural officials across the southern United States and throughout Mexico to detect the presence and monitor the spread and efficacy of control efforts. ARS developed and validated a sterile insect technique management/eradication program that was deployed by APHIS and the Mexican Secretary of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA-Mexico) as the foundation of the U.S.-Mexico Bi-National Cactus Moth Abatement Program. The sterile insect technique was used to eradicate the moth from various island infestations along coastal Alabama and Mississippi, and in Mexico's Yucatan Peninsula. Complete eradication of this pest from Mexico was declared in 2009, representing the first time a lepidopteran pest has been eradicated from a country in the Western Hemisphere. Progress has also been made in other management technologies with the discovery of a biological control agent for the cactus moth—the braconid wasp *Apanteles* sp., a new species with a narrow host range—and by using the moth's pheromone in mating disruption. Trials in Argentina have demonstrated moth suppression. APHIS acknowledged the success of the sterile insect technique program in controlling the cactus moth by honoring ARS scientists and colleagues with the APHIS-PPQ Deputy Administrator's Safeguarding Award in 2008.

Hight, S.D. and Carpenter, J.E. 2009. Flight phenology of male *Cactoblastis cactorum* (Lepidoptera: Pyralidae) at different latitudes in the southeastern United States. Florida Entomologist 92:208-216.

Tate, C.D., Hight, S.D., and Carpenter, J.E. 2009. Oviposition preference of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in caged choice experiments and the influence on risk assessment of F1 sterility. Biocontrol Science Technology 19(S1):317-333.

Sarvary, M.A., Bloem, K., Bloem, S., Carpenter, J., Hight, S., and Dorn, S. 2008. Diel flight pattern and flight performance of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) measured on a flight mill: the influence of age, gender, mating status and body size. Journal of Economic Entomology 101:314-324.

Sarvary, M.A., Hight, S.D., Carpenter, J.E., Bloem, S., Bloem, K.A., and Dorn, S. 2008. Identification of factors influencing flight performance of field-collected and laboratory-reared, overwintered, and nonoverwintered cactus moths fed with field-collected host plants. Environmental Entomology 37:1291-1299.

Bloem, K., Bloem, S., Carpenter, J., Hight, S., Floyd, J., and Zimmermann, H. 2007. Don't let cacto blast us – development of a bi-national plan to stop the spread of the cactus moth *Cactoblastis cactorum* in North America. Pp. 337-344. In M.J.B. Vreysen, A.S. Robinson, and J. Hendrichs (eds.). Area-Wide Control of Insects Pests: From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

PROBLEM STATEMENT IIIB: *Terrestrial, Aquatic, and Wetland Weeds*

Noxious weeds abound on public lands, rights-of-ways, and natural habitats throughout the United States. Most are invasive species and negatively impact these areas by displacing native species and altering entire ecosystems. Of the over 100 terrestrial and aquatic plants listed on the 2006 Federal noxious weed list, the vast majority were introduced, illustrating the critical need for research to develop improved border protection and mitigation strategies.

Under this problem statement, ARS scientists have sought to control invasive terrestrial and aquatic weeds, despite the enormous challenges because the weeds occur in a variety of habitats that often cover large areas. The scientists pursued biological and cultural approaches to manage the weeds, and if necessary integrated other best management practices, including the prudent use of herbicides. During this 5-year period, ARS scientists used classical biological control to successfully manage paperbark tree (*Melaleuca*); made significant progress toward managing a number of additional weeds, including Old World climbing fern, giant reed, saltcedar, tropical soda apple, leafy spurge, hydrilla, and water hyacinth; and identified candidate natural enemies for Chinese tallow and air potato. DNA fingerprinting methods have increased the efficiency of discovering biological control agents by allowing scientists to easily identify immature insects.

Biological control of the paperbark tree in Florida. In southern Florida, the invasive Australian paperbark tree (*Melaleuca quinquenervia)* develops monocultures in natural areas. The weed's environmental plasticity coupled with fire resistance, high reproductive potential, and deposition of large quantities of slowly degrading litter, lowers species diversity. ARS researchers in Fort Lauderdale, Florida, with collaborators at the ARS Australian Biological Control Laboratory in Brisbane discovered three biological control agents that eat the paperbark tree: a weevil (*Oxyops vitiosa*), a psyllid (*Boreioglycaspis melaleucae*), and a midge (*Lophodiplosis trifida*). These agents were rigorously tested for host-specificity and released on the trees in southern Florida, where they have established. Together with an adventive (from a different region and not yet fully established) rust fungus (*Puccinia psidii*), the released insects have defoliated, thinned the crowns, and killed paperbark trees. In 2011, an overall assessment of research sites showed a 30-fold decline in paperbark tree density, which translated into a nine-fold increase in native plant species richness. There was rapid re-colonization of vegetation gaps created by death of the paperbark trees, with the majority of re-colonizing species being members of native plant communities, e.g., sawgrass, dahoon holly, and wax myrtle. In addition, there has been a reduction in paperbark tree seedling recruitment due to lower seed quality and quantity. Since the biological control agents were introduced, paperbark tree monotypic landscapes are being progressively transformed into more diversified, healthier, plant communities. Together with herbicidal efforts by cooperators, the original 200,000 hectares invaded by the tree is now reduced to 100,000 hectares, with a 30 percent reduction in the Everglades, and acreage decline continues throughout the state. This is an example of successful classical biological control combined with integrated weed management. The total benefits of paperbark control in south Florida were estimated at $23.3 million. The biocontrol ratio for agricultural lands was 7.83 to 1. Also, agricultural managers reported lower costs since they have not been treating the paperbark tree as aggressively, and therefore have a greater marginal value at this stage of their control efforts. As a result of this research, land managers and private land owners are now equipped with the tools needed to implement a biologically-based paperbark tree management program on the lands they manage.

Center, T.D., Purcell, M.F., Pratt, P.D., Rayamajhi, M.B., Tipping, P.W., Wright, S.A., and Dray Jr., F. A. 2012. Biological control of *Melaleuca quinquenervia*: an Everglades invader. BioControl 57(2):151-165.

Tipping, P.W., Martin, M.R., Pierce, R. M., Center, T.D., Pratt, P.D., and Rayamajhi, M.B. 2012. Post-biological control invasion trajectory for *Melaleuca quinquenervia* in a seasonally inundated wetland. Biological Control 60:163-168.

Pratt, P.D., Rayamajhi, M.B., Center, T.D., Tipping, P.W., and Wheeler, G.S. 2009. The ecological host range of an intentionally introduced herbivore: A comparison of predicted versus actual host use. Biological Control 49:146-153.

Rayamajhi, M.B., Pratt, P.D., Center, T.D., Tipping, P.W., and Van, T.K. 2009. Decline in exotic tree density facilitates increased plant diversity: the experience from *Melaleuca quinquenervia* invaded wetlands. Wetlands Ecology and Management 17:455-467.

Franks, S.J., Pratt, P.D., Dray, F.A., and Simms, E.L. 2008. Selection on herbivory resistance and growth rate in an invasive plant. The American Naturalist 171(5):678-691.

Biological control of Old World climbing fern. Old World climbing fern *(Lygodium microphyllum)* is native to wet tropical and subtropical regions of Africa, Asia, Australia, and the Western Pacific Islands. In recent decades, the climbing fern has spread rapidly and infested large areas of southern and central Florida, where it competitively excludes native plants and provides an avenue for brush fires to reach into tree canopies. For this reason, the fern is regarded as one of the most serious invasive weeds affecting the state. ARS scientists in Fort Lauderdale, Florida, in collaboration with the ARS Australian Biological Control Laboratory in Brisbane and the Australian Commonwealth Science and Industrial Research Organization first discovered caterpillars of the moth *Neomusotima conspurcatalis* (Lepidoptera: Crambidae) feeding on the climbing fern in Hong Kong and western Australia**.** The moth was identified by ARS scientists in Beltsville, Maryland, and proven to be specific to the climbing fern and released by ARS scientists in Fort Lauderdale, Florida, into climbing fern dominated habitats in 2008. The moth is now well established, causing substantial damage to the weed. Following defoliation events, scientists have observed re-growth of the weed from dormant lateral buds. However the moth readily oviposits onto the re-growth and subsequent rounds of larval-caused defoliation occur on these patches. In 2008, the leaf-galling mite *Floracarus perrepae* was also found to be host-specific, released, and caused spectacular defoliation of the fern near Jupiter in south Florida; it is currently spreading naturally to new areas through controlled releases. Early indications suggest that feeding by the mite reduces development of the fern's sporangia and thereby decreases the weed's reproduction. While it is still too soon to predict the long-term impacts of the biological control agents, it is clear that the moth and mite play an important role in integrated control of this invasive weed. As an indication of support for this success, the Fort Lauderdale laboratory has received a grant of $26 million over 20 years to rear and redistribute these biological control agents via Florida resource managers, who have been able to reduce expenditures for herbicide treatment of the fern.

Boughton, A.J. and Pemberton, R.W. 2009. Establishment of an imported natural enemy, *Neomusotima conspurcatalis* (Lepidoptera: Crambidae) against an invasive weed, Old World climbing fern, *Lygodium microphyllum*, in Florida. Biocontrol Science and Technology 19:769-772.

Boughton, A.J., Bennett, C.A., Goolsby, J.A., and Pemberton, R.W. 2009. Laboratory host range testing of *Neomusotima conspurcatalis* (Lepidoptera: Crambidae): A potential biological control agent of the invasive weed, Old World climbing fern, *Lygodium microphyllum* (Lygodiaceae), Biocontrol Science and Technology 19:369-390.

An instrumented watershed approach for sustainable grazing and sage grouse habitat. Pinyon (*Pinus* spp.) and juniper (*Juniperus* spp.) occupy 19 million hectares in the U.S. West. Although pinyon and juniper are native tree species, changes in global climate, fire suppression, and

historic overgrazing have facilitated their encroachment into sagebrush steppe ecosystems, which have been reduced to 66 percent of 1860 levels. These evergreen, deeply-rooted trees compete with shrubs and grasses for limited water resources in semi-arid rangelands, thereby reducing associated valuable forage, soil stability, water availability, and fish and wildlife habitat, especially for the sage grouse that is a candidate for listing under the Endangered Species Act. In response to stakeholder concerns, ARS scientists in Reno, Nevada, in conjunction with scientists at the University of Nevada at Reno and Smith Creek Ranch, established the first instrumented watershed in Porter Canyon, Nevada, in 2009 to quantify how cutting these invasive trees would affect available water resources and understory plant community composition. Instrumentation was installed throughout a 6,000-acre watershed mostly managed by the Bureau of Land Management to measure all aspects of the water cycle including soil moisture, groundwater recharge, and water lost as evaporation and plant transpiration. In support of this project, the Bureau of Land Management conducted a regional environmental assessment that included the Porter Canyon Project. As part of this research, the private landowners harvested 56 hectares of trees and established two uncut 2-hectare research plots for ARS and the University of Nevada. The pinyon/juniper canopies prevented 44 percent of rainfall from reaching the soil. Total runoff did not differ as a function of plant foliar cover or slash cover. Sediment yield for plots with low foliar cover was 3.5 times greater than those with high foliar cover. Sediment yields for plots with slash presence were more than five times less than that of plots without slash. The width and depth of concentrated flow channels and sediment delivered to the bottom of the hill slope were significantly reduced as exposed bare ground was reduced as a direct benefit of the slash treatment. After 5 years of baseline data collected by ARS and the University of Nevada, the Bureau of Land Management will do further cutting of pinyon and juniper in the upper part of the watershed to improve habitat for the sage grouse, where they plan to measure watershed responses. To date, this is the first instrumented watershed in Nevada and this large-scale project has resulted in a national Bureau of Land Management conservation award to the landholder, Smith Creek Ranch (2010); fostered productive relationships with the Bureau, other agencies, and university partners; and, put ARS on the forefront of research designed to improve habitat for both the sage grouse and the sustainability of livestock grazing. This effort has resulted in numerous successful technology transfers and scientific findings. The research was featured on the National Public Broadcast service. ARS is now participating on the Pinyon-Juniper Steering Committee for the Great Basin and the Governor of Nevada's Sagebrush Ecosystem Technical Team for applying the best available science to management of sagebrush ecosystems. Results from Porter Canyon are be used by land managers to predict the response of similar watersheds to tree cutting treatments and for quantifying the environmental benefits derived from implementing conservation.

Lossing, S.S., Stringham, T.K., Carr, C.A., Snyder, K.A., and Weltz, M.A. Quantifying and modeling rainfall interception and redistribution by singleleaf piñon and Utah juniper. Journal of Arid Environments (in revision)

Completed Theses:

Dittrich, A.C. 2013. Summer water source partitioning by piñon and juniper: Quantifying water source and total water use by two Great Basin tree species in central Nevada. M.S. thesis submitted to University Nevada Reno, Reno, NV.

Lossing, S. 2012. Singleleaf pinon and Utah juniper canopy interception and understory characteristics in central Nevada. M.S. thesis submitted to University of Idaho, Moscow, Idaho.

Noelle, S.M. 2012. Effects if intercanopy vegetation cover and pinyon-juniper slash presenceon surface runoff and sediment production from steep slopes. M.S. thesis submitted to University of Arizona, Tucson, Arizona.

Costigan, K.H. 2010. Formation and evolution of concentrated flowpaths on a pinyon-juniper woodland. M.S. thesis submitted to University Nevada Reno, Reno, Nevada.

Invasive spread of swallow-worts revealed. Pale and black swallow-worts (*Vincetoxicum* spp.) are viny milkweeds that have become increasingly invasive in natural and managed habitats in the northeastern United States. As part of a biological control effort, ARS researchers in Ithaca, New York, in collaboration with Cornell University, are investigating the mechanisms of invasion and spread of these perennial weeds. Plant surveys taken in New York have shown that the swallow-wort's natural enemies from their native range in Europe are not present in the United States. In addition, ARS researchers have demonstrated that a phytochemical known as antofine, produced in the weed's roots and shoots, has potent antimicrobial activity and inhibits seedling growth of related native plant species, which are often present in the habitats invaded by these weeds. Experiments by the researchers have confirmed that swallow-wort seedling establishment, vegetative increase through the production of additional stems, and reproduction occur in both disturbed and non-disturbed environments and under a range of soil pH and light levels. The greatest increase and production of swallow-worts' wind-dispersed seeds is in open field environments, which likely serve as a primary source for new infestations of field and forest habitats. Based on this work, ARS and university researchers have recommended to land managers that management of these species in open fields should be a priority, especially as implementation of biological control will require several years.

Magidow, L.C., DiTommaso, A., Ketterings, Q.M., Mohler, C.L., and Milbrath, L.R. 2013. Emergence and performance of two invasive swallowworts (*Vincetoxicum* spp.) in contrasting soil types and soil pH. Invasive Plant Science and Management 6:281-291.

Averill, K.M., DiTommaso, A., Mohler, C.L., and Milbrath, L.R. 2011. Survival, growth, and fecundity of the invasive swallowworts (*Vincetoxicum rossicum* and *V. nigrum*) in New York State. Invasive Plant Science and Management 4:198-206 DOI:10.1614/IPSM-D-10-00034.1

Gibson, D.M., Krasnoff, S.B., Biazzo, J., and Milbrath, L. 2011. Phytotoxicity of antofine from invasive swallow-worts. Journal of Chemical Ecology 37:871-879. DOI:10.1007/s10886-011-9994-4.

Discovery, field release, and establishment of new natural enemies of giant reed in Texas. Giant reed (*Arundo donax*) is a highly invasive weedy grass from the Mediterranean region that displaces native riparian vegetation in the United States and clogs waterways in Southern border States wasting precious water in these dry regions. Its dense thickets also hinder effective border patrol activities and provide habitat for the tick that carries cattle fever. Giant reed became a problem in the United States because it lacks effective natural enemies. Scientists at the ARS European Biological Control Laboratory in France have now identified four candidate natural enemies after making more than 250 field collections in Spain, France, Italy, and Greece. The candidates were shipped to U.S. quarantine facilities in Mission, Texas, where ARS scientists evaluated them for safety and efficacy against the weed. Two of the agents, a gall-forming wasp *(Tetramesa romana)* and a scale insect *(Rhizaspidiotus donacis)*, have received APHIS release permits and have been released into the field. A third agent, a leaf-

mining fly (*Lasioptera donacis*), is currently being evaluated in quarantine. ARS scientists in Kerrville, Texas, have made significant advances in understanding the biological association of the defoliating leafminer fly and associated endophytic pathogens that will help determine the role of the leafminer-pathogen complex in biological control. The arundo wasp and arundo scale are now well-established throughout the bi-national Rio Grande Basin below Del Rio, Texas. Arundo wasp populations reached damaging levels all along the Rio Grande during 2012 which caused thinning of giant reed stands and increased visibility of the border, which is of great importance to the U.S. Border Patrol. The arundo scale is beginning to reduce recruitment of new reeds where it is established in Del Rio. ARS scientists are developing methods to mass rear and aerially release the biological control agents on the Rio Grande and other areas affected by this invasive weed. As these natural controls spread, they will help to suppress the grass and restore original riparian habitats. A comprehensive evaluation plan is underway to quantify the benefits to stakeholders including, cattle producers, agriculture and municipal water districts, and the Border Patrol.

Tarin, D., Manhart, J., Pepper, A., Goolsby, J., Moran, P., Contreras-Arquieta, B., and Kirk, A. 2013. Microsatellite markers indicate multiple origins of *Arundo donax* L. in North America. Invasive Plant Science and Management 6:328-338.

Yang, C., Everitt, J.H., and Goolsby, J.A. 2011. Using aerial photography for mapping giant reed infestations along the Texas-Mexico portion of the Rio Grande. Invasive Plant Science and Management 4:402-410.

Goolsby, J.A., Moran, P.J., Adamczyk, J.A., Kirk, A.A., Jones, W.A., Marcos, M., and Cortés, E. 2010. Host range of the European, rhizome-stem feeding scale *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae), a candidate biological control agent for giant reed, *Arundo donax* L. (Poales: Poaceae) in North America. Biocontrol Science and Technology 19:899-918.

Goolsby, J.A., Spencer, D., and Whitehand, L. 2010. Pre-release assessment of impact on *Arundo donax* by the candidate biological control agents, *Tetramesa romana* (Hymenoptera: Eurytomidae) and *Rhizaspidiotus donacis* (Homoptera: Diaspididae) under quarantine conditions. Southwestern Entomologist 34:359-376.

Moore, G.W., Watts, D.A., and Goolsby, J.A. 2010. Ecophysiological Responses of Giant Reed (*Arundo donax*) to Herbivory. Invasive Plant Science and Management 3:521-529.

Racelis, A.E., Goolsby, J.A., Penk, R., Jones, W.K., and Roland, T.J. 2010. The development of an inundative, aerial release technique for the arundo wasp, a biological control agent of the invasive *Arundo donax*. Southwestern Entomologist 35:495-501.

Seawright, E.K., Rister, M.E., Lacewell, R.D., McCorkle, D.A., Sturdivant, A.W., Yang, C. and Goolsby, J.A. 2010. Economic implications for the biological control of *Arundo donax* in the Rio Grande Basin. Southwestern Entomologist 34:377-394

Invasive guineagrass in the United States is of hybrid origin. Over the last several decades invasive grasses have become increasingly prevalent. One of these, guineagrass, which is native to Africa, had been introduced as a source of animal fodder throughout tropical areas of both hemispheres. Guineagrass is becoming a serious threat to global biodiversity, not only due to it crowding out native species, but also because it depletes surface water and produces high biofuel loads that promote wildfires. It is highly invasive in natural areas, highway right-of-ways, and in

crops such as citrus and sugarcane. Herbicidal, mechanical, and cultural controls are expensive and largely ineffective. ARS scientists in Weslaco, Texas, and the European Biological Control Laboratory in France collected a large number of natural enemies of guineagrass in Cameroon, where the weed is common. Noting that the morphology of African and Texan guineagrass differs significantly, the scientists analyzed sequences of maternally and biparentally inherited markers. The analyses indicated that the Texas populations are actually allopolyploids (i.e., having two or more complete sets of chromosomes derived from different species), in which one of the two diploid parental donors naturally occurs in eastern Africa. Furthermore, guineagrass in Mexico and Central and South America is a different species from the guineagrass in Texas. This is important information suggesting that the Mexico species (*Megathrysus maximus*) used for forage will not be harmed by release of biocontrol agents against the Texas species (*M. infestus*). These results will be instrumental in guiding future exploration and collection of appropriate biotypes of natural enemies of guineagrass in Africa.

Mercadier, G., Goolsby, J.A., Jones, W.A., and Tamesse, J.L. 2009. Results of a preliminary survey in Cameroon, Central Africa, for potential natural enemies of guineagrass, *Panicum maximum,* Subtropical Plant Science 61:31-36.

Discovery of genetic diversity in flea beetles used in leafy spurge biocontrol. Leafy spurge infests about 1 million acres in the plains and foothills of central North America, and *Aphthona* flea beetles, introduced into the United States (and Canada), are the primary means of control in non-cultivated areas (e.g., rangeland and national forests). Because genetic diversity is one factor that could have an overall effect on *Aphthona* establishment in specific locations, ARS scientists in Fargo, North Dakota, with university, State, and industry collaborators, catalogued the genetic variation in established flea beetle populations. Five species of flea beetles have been successfully introduced into the United States, and ARS scientists have developed a molecular marker key to identify both adults and immature stages of these species. Using these markers, ARS scientists discovered populations of two *Aphthona* species (*A. czwalinae and A. flava*) in North Dakota; whereas, over the last decade, these species had been believed to be absent from the State. The markers also identified large populations of *A. cyparissiae* in west-central Minnesota previously unrecognized. The identification key and procedures developed are available for use by scientists tracking the movement and effectiveness of these biocontrol beetles. Without the marker assay, these populations could not have been characterized as successful introductions. Further, detailed phylogenetic analysis of mitochondrial DNA (mtDNA) by the ARS scientists revealed that two of the flea beetle species have distinct genetic subpopulations. *A. nigriscutis* is divided into two mtDNA groups, one of which is infected with a symbiotic *Wolbachia* bacterium that drastically reduces its number of males. The association of *Wolbachia* with one, but not both, mtDNA groups of *A. nigriscutis* may play a role in limiting genetic diversity within that species. Because *A. nigriscutis* is such an important biocontrol agent of leafy spurge, any factors that may affect its establishment and reproductive success are of potential importance. *A. lacertosa* comprises three mtDNA groups, two of them unique to Canada, which raises the possibility that Canadian and U.S. releases of insects identified as *A. lacertosa* may have been more than one species. Whether they are different species or discrete genetic variants of *A. lacertosa*, the control program might benefit from greater dispersal of these variants. Such information is essential for determining whether new natural enemies need to be established, as well as providing important clues on where to look for additional agents as needed.

Roehrdanz, R., Bourchier, R., Cortilet, A., Olson, D., and Sears, S. 2011. Phylogeny and genetic diversity of flea beetles (*Aphthona* sp.) introduced to North America as biological control agents for leafy spurge. Annals of Entomological Society of America 104:966-975.

Roehrdanz, R., Olson, D., Fauske, G., Bourchier, R., Cortilet, A., and Sears, S. 2009. New DNA markers reveal presence of *Aphthona* species (Coleoptera: Chrysomelidae) believed to have failed to establish after release into leafy spurge. Biological Control 49:1-5.

Production of the bioherbicide Mycoleptodiscus terrestris for managing aquatic weed infestations. Hydrilla (*Hydrilla verticillata*) and Eurasian watermilfoil (*Myriophyllum spicatum*) are invasive aquatic weeds that are major threats to aquatic ecosystems in the United States and around the world. ARS researchers in Peoria, Illinois, developed the first liquid culture fermentation method for the production of high concentrations of a stable, infective form of the aquatic fungus *Mycoleptodiscus terrestris* that kills hydrilla, Eurasian watermilfoil, and a number of other aquatic weeds. The ARS researchers partnered with the U.S. Army Corps of Engineers to apply this technology to alleviate hydrilla infestations clogging waterways in the southeastern United States. U.S. and foreign patents have been issued on this novel composition of the stable infective form of *M. terrestris* and were licensed to a commercial partner. Eurasian watermilfoil management trails in Michigan are ongoing. The success of this technology resulted in the Federal Laboratory Consortium Award for Excellence in Technology Transfer in 2010.

Dunlap, C.A., Jackson, M.A., and Saha, B.C. 2011. Compatible solutes of sclerotia of Mycoleptodiscus terrestris under different culture and drying conditions. Biocontrol Science and Technology 21:112-123.

Jackson, M.A., Dunlap, C.A., Shearer, J.F., and Heilman, M.A. 2011. The impact of temperature on the production and fitness of microsclerotia of the fungal bioherbicide *Mycoleptodiscus terrestris*, a pathogen of the aquatic weed hydrilla. Biocontrol Science and Technology 21:547-562.

Jackson, M.A. and Shearer, J.F. 2008. Mycoherbicidal compositions and methods of preparing and using the same. Canadian Intellectual Property Office, Patent no. 2,485,231.

Jackson, M.A. and Shearer, J.F. 2003. Mycoherbicidal compositions and methods of preparing and using the same. IP Australia, Patent no. 2003299477.

Integrated management of invasive Ludwigia hexapetala. Invasive species are a major threat to the National Wildlife Refuge System; in 2011, the U.S. Fish and Wildlife Service reported that more than 2.4 million acres of refuge lands were affected by invasive plants. While the National Wildlife Refuge System's invasive weed management expenditures have risen to $16.5 million per year**,** these resources can only treat approximately 10 percent of the infested acreage in the United States. *Ludwigia hexapetala* (Uruguayan primrose-willow) is an invasive aquatic plant that forms buoyant mats over the water surface and crowds or shades out natural species of water plants in canals, rivers, and other wetlands in the Pacific West. ARS scientists in Davis, California, partnering with U.S. Fish and Wildlife Service managers, used hydrologic manipulation, physical removal, and herbicide application to develop successful integrated management methods for control of the weed. The methods led to improved efficacy of control in irrigation canals, cost reductions, and a decrease in herbicide reliance. The scientists also tested the timing and frequency of dry season tillage for control of the weed in managed seasonal wetlands. Disking at early flowering stage improved control and reduced emergence of the weed

from soil seed banks. ARS scientists provided their findings in presentations at workshops for State, Federal and private marsh managers throughout California and, in cooperation with the Oregon Department of Agriculture, presented results to agricultural and natural lands managers at annual noxious weed management symposia in Oregon. To date, these efforts have improved the effectiveness of invasive *Ludwigia* control in irrigation district canals, the 35,000-acre Sacramento National Wildlife Refuge complex, and on impacted wetlands in 30,000 acres of private Sacramento Valley lands in the Federal conservation easement program. Control methods are now being adopted in the Willamette Valley, Oregon. ARS scientists are also evaluating invasive traits of the weed and, with collaborators, have proposed a new framework to evaluate these traits in changing environments as a basis for improved restoration and management of a variety of habitats.

Drenovsky, R.E., Grewell, B.J., D'Antonio, C.M., Funk, J.L., James, J.J., Molinari, N., Parker, I.M., and Richard C.L. 2012. A functional trait perspective on plant invasion. Annals of Botany 110:141-153.

Hoch, P.C. and Grewell B.J. 2012. Ludwigia. (Onagraceae) *in:* B.G. Baldwin (conv. ed.) The Jepson Manual; Higher Plants of California, Second Edition. University of California Press, Berkeley, California, pp. 948-949.Carruthers, R.I., Franc, M.K., Gee, W.S., Cosse, A.A., Grewell, B.J., and Beck, J.J. 2011. Volatile emissions from the flea beetle *Altica litigata* (Coleoptera: Chrysomelidae) associated with invasive *Ludwigia hexa*petala. Chemoecology 21:253-259.

Okada, M., Grewell, B.J., and Jasieniuk, M. 2009. Clonal spread of invasive *Ludwigia hexapetala* and *L.grandiflora* in freshwater wetlands of California. Aquatic Botany 91:123-121.

Perennial pepperweed control for endangered species recovery. Perennial pepperweed (*Lepidium latifolium*), native to Eurasia, is a major weed in western U.S. States that has recently spread throughout the Sacramento-San Joaquin Delta and the greater San Francisco Estuary where it reduces forage quality in hay or pasture and native plant and animal species in tidal wetlands. The Delta is the heart of State and Federal water project operations in California, and many weed management proposals in habitat occupied by endangered species have been delayed due to concerns about the potential non-target effects of treatments to control the weed. To expedite management efforts, ARS scientists in Davis, California, have partnered with the U.S. Fish and Wildlife Service and the California Department of Parks and Recreation to test perennial pepperweed control strategies for native and endangered plant recovery in the estuary. In Suisun Marsh, California, field experiments tested the efficacy of two herbicides: 2,4-D, which showed minimal non-target effects, but did not provide effective control; and imazypry, which reduced pepperweed cover by 90 percent, but had persistent non-target effects on native vegetation. At a State park preserve, ARS scientists evaluated the spatial and temporal distribution and abundance of pepperweed and rare plants it affects as part of a marsh-wide adaptive management program for a long-term pepperweed control field project. The scientists established stratified treatment strategies, evaluated the efficacy of hand-wick and backpack sprayer applications of aquatic glyphosate, and tested demographic responses of the rare plants to weed management actions. Pepperweed biomass and density were significantly reduced by herbicide treatment. Following 2 years of treatment, 71 percent of remaining pepperweed had been reduced from high to low cover class, while the occupied area of the endangered plant increased by 24 percent with a 211 percent increase in the number of occupied rare plant patches.

Results demonstrate that careful, science-based adaptive management can be successful for invasive weed suppression in highly sensitive endangered species habitat. Both the U.S. Fish and Wildlife Service and the California Department of Parks and Recreation have adopted the ARS science-based approach as a model for adaptive weed management and endangered species recovery.

Berkeley, C.A., Whitcraft, C.R., and Grewell, B.J. 2011. Evaluation of perennial pepperwood management in a seasonal wetland in the San Francisco Estuary prior to restoration of tidal hydrology. Wetland Ecology and Management 20:35-45.

Whitcraft, C.R, Grewell, B.J., and Baye, P.R. 2011. Estuarine vegetation at Rush Ranch Open Space Preserve, San Francisco Bay National Estuarine Research Reserve, California. San Francisco Estuary and Watershed Science 9(3):1-29.

Grewell, B.J. 2008. Hemiparasites generate environmental heterogeneity and enhance species coexistence in salt marshes. Ecological Applications 18(5):1297-1306.

Grewell, B.J. 2008. Parasite facilitates plant species coexistence in a coastal wetland. Ecology 89:1481-1488.

Saltcedar biological control developed along the Rio Grande and other Texas rivers. Non-native (from Tunisia and Greece), invasive saltcedar has colonized over 500 river-miles along the Rio Grande with similar populations on other Texas rivers. Saltcedar reduces natural plant and animal biodiversity, consumes precious water resources, and increases soil salinity, while also producing phenolic resins that boost fire danger. ARS scientists in Temple and Weslaco, Texas, released saltcedar leaf beetles (*Diorhabda elongata* and *D. sublineata*) in subtropical south Texas, while NRCS and cooperators at the U.S. National Park Service released the beetles in southwestern Texas along the Rio Grande. ARS scientists then evaluated the effects of the saltcedar beetles on both saltcedar and athel, a close non-native (invasive) relative that provides some benefits as shade and windbreak in its native Mexico. The beetles did not establish populations in subtropical South Texas and did no damage to athel. In the southwestern Big Bend region, the beetles removed all foliage from saltcedar and dispersed over 150 miles in 3 years. The beetles defoliated large athel trees along the Rio Grande in 2010, but the trees recovered within 1-3 months, with little-to-no beetle damage to athel in subsequent years. Meanwhile, saltcedar trees have been repeatedly defoliated. On the basis of these results, Mexican agencies agreed to allow release of beetles on the U.S. side of the Rio Grande, and continued monitoring of biological control impacts. Released saltcedar beetles on the Pecos, Upper Colorado, and other Texas rivers by Texas A&M University scientists and others have caused large-scale defoliation of saltcedar and recovery of native vegetation.

Moran, P.J. 2010. Lack of establishment of the Mediterranean tamarisk beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae) on athel (*Tamarix aphylla*) (Tamaricaceae) in South Texas. Southwestern Entomologist 35:129-145.

Moran, P.J., DeLoach, C.J., Dudley, T.L., and Sanabria, J. 2009. Open field host selection and behavior by tamarisk beetles (*Diorhabda* spp.) (Coleoptera: Chrysomelidae) in biological control of exotic saltcedars (*Tamarix* spp.) and risks to non-target athel (*T. aphylla*) and native *Frankenia* spp. Biological Control 50:243-261.

Elucidating the ecosystem effects of insect biological control. Riparian environments such as wetlands and river or stream corridors occupy a small fraction of the landscape in arid western U.S. rangelands, yet are tremendously important in terms of the ecosystem services they provide. Woody species of the genus *Tamarix* (saltcedar), which range in stature from large shrubs to small trees, have invaded many of these riparian systems, replacing native woody plants, such as cottonwoods and willows, and raising concerns regarding multiple environmental issues, especially the impact of saltcedar on water-use potential and wildlife populations and habitat. The importance and complexity of addressing these issues are relevant since many saltcedar stands are experiencing rapid changes due to ongoing biological control efforts relating to the release and spread of saltcedar leaf beetles, *Diorhabda* spp. ARS scientists in Reno, Nevada, are conducting long-term studies of the impact of saltcedar invasions on both ecosystem function (carbon, water, and nutrient cycling) and wildlife, and addressing impacts of biological control on these environmental issues. The majority of research on the impacts of saltcedar on wildlife has focused on bird populations and communities. In the first comprehensive study of saltcedar impact on small mammals, the researchers showed in a 12-year data set that the species composition of riparian environments experienced subtle changes when saltcedar invaded. Some mammal species that occur in both native and saltcedar habitats maintained superior body condition in the former. This suggests that successful control of saltcedar may benefit local small mammal communities, which supports continuation of biocontrol efforts against the weed. In addition, another multi-year dataset was developed using a state-of-the-art eddy-covariance tower to measure evapo-transpiration and carbon dioxide exchange at the stand level. These data demonstrated that, during the first 4 years of beetle herbivory, the impact of herbivory on saltcedar physiology did not substantially reduce ecosystem water loss or affect net carbon storage. However, beetle herbivory substantially changed the quality and quantity of leaf litter in the short-term, which may have implications for longer term nutrient cycling.

Uselman, S.M., Snyder, K.A., and Blank, R.R. 2013. Impacts of insect biological control on soil N transformations in Tamarix-invaded ecosystems in the Great Basin, NV. Journal of Arid Environments 88:147-155.

Longland, W.S. 2012. Small mammals in saltcedar (*Tamarix ramosissima*)-invaded and native riparian habitats of the western Great Basin. Invasive Plant Science and Management 5:230-237.

Uselman, S.M., Snyder, K.A., and Blank, R.R. 2011. Insect biological control accelerates leaf litter decomposition and alters short-term nutrient dynamics in a *Tamarix*-invaded riparian ecosystem. Oikos 120:409-417.

Uselman, S.M., Snyder, K.A., Blank, R.R., and Jones, T.J. 2011. UVB exposure does not accelerate rates of litter decomposition in a semi-arid riparian ecosystem. Soil Biology and Biochemistry 43:1254-1265.

Hultine, K.R., Belnap, J., van Riper, C., Ehleringer, J.R., Dennison, P.E., Lee, M. E., Nagler, P.L., Snyder, K.A., Uselman, S.M., and West, J.B. 2010. Tamarisk biocontrol in the western United States: ecological and societal implications. Frontiers in Ecology and the Environment 8:467-474.

Snyder, K.A., Uselman, S.M., Jones, T.J., and Duke, S. 2010. Ecophysiological responses of salt cedar (*Tamarix* spp. L.) to the northern tamarisk beetle (*Diorhabda carinulata* Desbrochers) in a controlled environment. Biological Invasions 12:3795-3808.

New biological agents and strategies for control of waterway weeds. Waterhyacinth, *Eichhornia crassipes*, remains the world's most troublesome aquatic weed and continues to invade water bodies and wetlands in new regions. Waterhyacinth blankets large water bodies creating impenetrable barriers which affect water traffic, water quality, access to water, and biodiversity, while blocking access to recreational areas and harming the economies of communities that depend upon fishing and water sports for revenue. Waterhyacinth and Brazilian waterweed, *Egeria densa*, which also contributes to choked waterways, are found in all U.S. Gulf Coast states, in the San Joaquin and Sacramento River Delta in California, and extend southward through Mexico. Herbicidal treatment or mechanical harvesting of these weeds is expensive, often damages nearby desirable vegetation, and must be applied in perpetuity. ARS scientists in Fort Lauderdale, Florida, and at the ARS South American Biological Control Laboratory in Buenos Aires, Argentina, have discovered and deployed *Megamelus scutellaris* (Hemiptera: Delphacidae), the first biological control agent released against waterhyacinth in more than 30 years. This highly mobile insect develops rapidly, has demonstrated complete fidelity to waterhyacinth, and has experimentally reduced waterhyacinth biomass by greater than 66 percent in only 2 months. Thousands of insects have been transferred to Federal, State, and local cooperators in five States to begin the process of sustainably suppressing waterhyacinth to restore millions of acres of freshwater ecosystems. The scientists have also evaluated and gotten approval for importation of additional biocontrol agents against waterhyacinth and waterweed for testing. And, the scientists are evaluating another strategy of using waterhyacinth weevils for biological control before herbicide use to shorten the response of the herbicide by 1 week. This suggests a strategy for weed management suited to areas where the biocontrol agents do not yet provide adequate control. ARS scientists in Albany and Davis, California, have been providing data to aid California in obtaining Federal permits to control these invasive species in areas where endangered species are known to exist. Through these efforts, integrated control programs have been implemented annually over many important waterways

Cabrera Walsh, G.Y., Dalto, M., Mattioli, F.M., Carruthers, R.I., and Anderson, L.W. 2013. Biology and ecology of Brazilian elodea (*Egeria densa*) and it specific herbivore, *Hydrellia* sp., in Argentina. BioControl 58:133-147.

Fitzgerald, D. and Tipping, P.W. 2013. Effect of insect density and host plant quality on wing-form in *Megamelus scutellaris* (Hemiptera: Delphacidae). Florida Entomologist 65:124-130.

Moran, P.J. 2012. Influence of biological control damage on efficacy of penoxsulam and two other herbicides on waterhyacinth. Journal of Aquatic Plant Management 50:32-38.

Tipping, P.W., Center, T.D., Sosa, A.J., and Dray Jr., F.A. 2011. Host specificity assessment and potential impact of *Megamelus scutellaris* (Hemiptera: Delphacidae) on waterhyacinth *Eichhornia crassipes* (Pontederiales: Pontederiaceae). Biocontrol Science and Technology 21:75-87.

Interactions of native parasitoids and insect biological control agents selected for weed control. The weed, yellow starthistle, *Centaurea solstitialis* (Asteraceae), is recognized as one of the top five weeds in the western United States. Biological control was initiated in 1990 with the release of the first agent. However, this agent was so heavily parasitized that between 1984 and 1994, five additional insect seed predators were introduced, which also have not satisfactorily controlled the weed. In 2003, a fungal biocontrol pathogen was introduced

throughout California, but it appears to have a very narrow climatic zone and has failed to establish anywhere except in the wettest coastal areas. For herbivores, the problem that researchers face is that often it is not known how native species, particularly parasites and predators, alter the impact of the agent on its target weed. To explore this question, ARS scientists in Reno, Nevada, used extensive field data sets, including a multi-year, multi-site demographic study of yellow starthistle. Mortality of the biocontrol agent (*Eustenopus villosus*, hairy weevil) was determined by dissecting more than 6,000 infested inflorescences to determine percent parasitism. The extensive field data sets were coupled with a modeling approach, showing that native generalist parasitoids can reduce and even eliminate the impact of a potentially, very effective biocontrol agent on its target weed. Such an outcome is impossible to predict from simple pair-wise tests of agents on weeds. These data indicate that an additional type of host testing may be necessary prior to agent approval and release, one that investigates susceptibility of the agent to attack by native parasitoids/predators. Specific recommendations for new tests would minimize the risk of host switching by generalist parasitoids and increase the likelihood of biocontrol success. These recommendations are to avoid herbivorous biocontrol agents that are expected to be heavily parasitized in the introduced range due to the presence of closely related native herbivorous species, agents that are heavily parasitized in their native range, and agents that feed on plants in a manner similar to native insects. This strategy would make biological control more cost effective since only agents that are most likely to reduce weed abundance would be released.

Swope, S.M. and Satterthwaite, W.H. 2012. Variable effects of a generalist parasitoid on a biocontrol seed predator and its target weed. Ecological Applications 22(1):20-34.

Molecular genetics used to assist evaluation of host plant specificity for biological control. Prospective biological control agents are assessed for host plant specificity to minimize the risk of introducing agents that might damage non-target plants. However, the artificial conditions of laboratory trials sometimes result in an insect attack on non-target plants that would not normally occur under field conditions. ARS scientists in Albany, California, conducted field and laboratory experiments to measure the host plant specificity of a weevil, *Ceratapion basicorne*, that develops inside the root crown of yellow starthistle. Because several closely related insect species attacked the plants in some study sites, it was critical to identify which insect species were present on yellow starthistle and the crop safflower, a related plant. The scientists developed methods to identify the insect species using PCR-based molecular genetic analysis of immature insects, which was necessary because plants had to be harvested before adults emerged, and only adults could be identified by morphological methods. The results of a series of seven field experiments confirmed that safflower was not attacked by the weevil, despite the fact that this plant was sometimes attacked under laboratory conditions where the weevil was severely confined with safflower. The results are being submitted to APHIS to support an application for a permit to release the agent.

Smith, L. 2012. Host plant oviposition preference of *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of yellow starthistle. Biological Control Science and Technology 22(4):407-418.

Rector, B.G., De Biase, A., Cristofaro, M., Primerano, S., Belvedere, S., Antonini, G., and Sobhian, R. 2010. DNA fingerprinting to improve data collection efficiency and yield in an open-field host-specificity test of a weed biological control candidate. Invasive Plant Science and Management 3(4):429-439.

Antonini, G., Coletti G., Serrani, L., Tronci, C., Cristofaro, M., and Smith, L. 2009. Using molecular genetics to identify immature specimens of the weevil *Ceratapion basicorne* (Coleoptera, Apionidae). Biological Control 51:152-157.

Managing phosphorus to control algal blooms in rice fields. Inorganic phosphorus is applied as a fertilizer to enhance rice seedling growth. In recent years there has been a shift from incorporating this material during field preparation to applying it directly on the soil surface. As a result, the inorganic phosphorus dissolves in the initial water used to flood the field and is available to support excessive algal growth, especially that of the cyanobacterium *Nostoc spongiaeforme*. ARS scientists in Davis, California, demonstrated that algae blooms detrimental to rice yields are caused by high levels of inorganic phosphorus in rice field water. The scientists and colleagues from the University of California at Davis demonstrated that this problem could be addressed by delaying application of phosphorus until 30 days after initial field flooding without loss of rice yield. This practice has been adopted by rice growers and has resulted in reduced algal growth in rice fields, as well as a reduced need to apply traditionally used algicides. This approach has been highlighted and recommended in several agriculturally related popular press articles, including the weekly newspaper for California Agriculture and the publication Rice Farming.

Spencer, D.F., Liow, P-S., and Lembi, C.A. 2013. Influence of a non-copper algicide on the cyanobacterium, *Nostoc spongiaeforme*, and the green algal, *Hydrodictyon* sp., in field and laboratory experiments. Paddy and Water Environment 11:611-617. DOI:10.1007/s10333-012-0343-1.

Lundy, M.E., Spencer, D.F., Van Kessel, C., Hill, J.E., and Lindquist, B.A. 2012. Managing phosphorus fertilizer to reduce algae, maintain water quality, and sustain yields in water-seeded rice. Field Crops Research 131:81-87.

Spencer, D.F., Liow, P-S., and Lembi, C.A. 2009. Effect of a combination of two rice herbicides on the cyanobacterium, *Nostoc spongiaeforme*. Journal of Aquatic Plant Management 47:145-147.

Spencer, D.F. and Lembi, C.A. 2007. Evaluation of barley straw as an alternative algal control method in California rice fields. Journal of Aquatic Plant Management 45:84-90.

Biological control combined with burning or mowing for management of Scotch broom. The invasive weedy legume Scotch broom, *Cytisus scoparius*, is mildly toxic and unpalatable to most animals except goats. It forms dense stands that reduce wildlife habitat and competes with seedling trees in reforestation efforts, costing Oregon alone $47 million per year in lost timber production. Individual broom plants in Fort Lewis, Washington, produced around 30,000 seeds, 3-6 times more seeds than it produces in its native range. ARS scientists in Albany, California, in cooperation with the U.S. Army, The Nature Conservancy, and the University of California at Berkeley, tested a combination of burning, mowing, and the use of insect natural enemies over a 10-year period to assess the best methods for controlling the weed on rangeland across Fort Lewis. Results showed that biological control of broom in combination with either burning or mowing reduced both the number of seed pods per plant and the number of viable seeds per pod, lowering seed bank densities by 82 percent. The Nature Conservancy now implements this combined broom management strategy in cooperation with the Fort Lewis Environmental

Management Team across all range areas. For Scotch broom, biological control is now a background activity requiring no cost or special actions, while both fire and mowing are scheduled based on available funding.

Herrera-Reddy, A. M., Carruthers, R. I., and Mills, N.J. 2012. Integrated management of Scotch broom (*Cytisus scoparius*) using biological control. Invasive Plant Science and Management 5(1):69-82.

New approach to improve safety assessments of weed biological control pathogens. Biocontrol agents must be tested for non-target effects before being released. To facilitate and increase the validity of this testing, ARS researchers in Frederick, Maryland, developed a mixed model statistical approach, incorporating information on genetic relationships among target and non-target plant species with data on disease reactions to get broad-based predictions of the susceptibility of each plant species to three promising microbial control agents. An added value of this approach is the ability to predict disease reactions on species that cannot be adequately tested because the pathogens are either very rare or extremely difficult to grow. Based on this study, each of the pathogens has been deemed safe to non-target plants and damaging to the target weeds on the basis of greenhouse tests. The approach has culminated in petitions for release of three pathogens to the Technical Advisory Group of APHIS-PPQ. Proposals for *Boeremia exigua* var. *rhapontica* (vs. Russian knapweed, *Rhaponticum repens*) and *Ramularia crupinae* (vs. two varieties of common crupina, *Crupina vulgaris*) are presently before the Technical Advisory Group, and one for the rust *Uromyces salsolae* vs. the tumbleweed Russian thistle (*Salsola tragus*) has been approved and is under review by APHIS-PPQ regulators.

Berner, D.K. and Bruckart, W.L. 2012. Comparing predictions from mixed model equations with host range determinations from historical disease evaluation data of two previously released weed biological control pathogens. Biological Control 60:207-215.

Berner, D.K. and Cavin, C.A. 2012. Finalizing host range determination of a weed biological control pathogen with best linear unbiased predictors and damage assessment. BioControl 57:235-246.

Berner, D.K. 2010. BLUP, a new paradigm in host-range determination. Biological Control 53:143-152.

Berner, D.K., Bruckart, W.L., Cavin, C.A., and Michael, J.L. 2009. Mixed model analysis combining disease ratings and DNA sequences to determine host range of *Uromyces salsolae* for biological control of Russian thistle. Biological Control 49:68-76.

Ecosystem-based approach to invasive annual grass management. Conservatively, at least 130 million acres of the Great Basin are infested with invasive annual grasses, and the permanent conversion of a once-diverse landscape to one limited to invasive grasses is of critical concern. Once ecosystems have been severely degraded, it is extremely difficult to restore and re-vegetate, with some estimates of success as low as 5 percent. Traditionally, rangeland weed managers focused efforts on killing weeds, rather than addressing the underlying causes of invasion. Nevertheless, to achieve sustainable invasive weed management, managers would benefit from knowing how to modify the underlying ecological processes that control favorable vegetation dynamics. ARS scientists in Burns, Oregon, have developed and tested an ecological framework based on modifying ecological processes to manage invasive annual grasses. The framework—Ecologically Based Invasive Plant Management (EBIPM)—combines state and

transition models and successional management as a framework to make the best management decisions for a given landscape based on ecological principles. It has been adopted and currently is being used to manage these grasses in several western states. Adopted by over 3,000 land managers and producers, EBIPM is now used to manage over 5 million acres. The success of this research resulted in two top awards, including the 2012 ARS Superior Technology Transfer Award and the 2012 National Federal Laboratory Consortium for Technology Transfer Award for Excellence in Technology Transfer.

Sheley, R.L., James, J.J., Vasquez, E.A., and Svejcar, T.J. 2011. Using rangeland health assessment to inform successional management. Invasive Plant Science and Management (3):356-366.

James, J.J., Smith, B. S., Vasquez, E.A., and Sheley, R.L. 2010. Principles for ecologically based invasive plant management. Invasive Plant Science and Management 3(3):229-239.

Sheley, R., James, J., Smith, B., and Vasquez, E. 2010. Applying ecologically based invasive-plant management. Rangeland Ecology and Management 63(6):605-613.

Biological control of air potato. Air potato, *Dioscorea bulbifera*, is native to tropical and subtropical Asia and Africa. This weed seldom flowers in the United States and reproduces primarily through aerial bulbils (hence, the common name air potato), vegetative propagules that form in the leaf axils and dehisce as the plant senesces in the fall. This herbaceous, perennial vine is found in the southeastern United States, Puerto Rico, Texas, and Hawaii. Air potato vines grow more than 20 meters long, climb into trees, and disrupt natural areas by outcompeting native vegetation, which is often blanketed by the vines. In Florida, this plant invades a variety of habitats and threatens native ecosystems in ecologically sensitive preserves, as well as urban parks. ARS scientists in Fort Lauderdale, Florida, discovered the chyrsomelid beetle *Lilioceris cheni* feeding on air potato in Nepal. Host-range testing by the scientists demonstrated that the beetle is specific to *D. bulbifera* and a release permit was obtained in 2011. Open field releases of the beetle commenced in 2012, and to date nearly 39,000 beetles have been released in 18 counties in Florida. Preliminary results indicate that beetle populations increase rapidly in the field and are capable of causing extensive defoliation and premature leaf drop of the weed, which decreases the reproductive capacity of the vine by limiting bulbil production.

Center, T.D., Rayamajhi, M., Dray, F.A., Madeira, P. M., Witkus, G., Rohrig, E., Mattison, E., Lake, E., Smith, M., Zhang, J., Purcell, M., Konstantinov,A., and Schmitz, D. 2013. Host range validation, molecular identification, and release and establishment of a Chinese biotype of the Asian leaf beetle *Lilioceris cheni* (Coleoptera: Chrysomelidae: Criocerinae) to control of *Dioscorea bulbifera* L. in the southern United States. Biocontrol Science and Technology 23(7):735-755.

Pemberton, R.W. and Witkus, G.L. 2010. Laboratory host range testing of *Lilioceris* sp. Near *impressa* (Coleoptera: Chrysomelidae) – a potential biological control agent of air potato, *Dioscorea bulbifera* (Dioscoreaceae). Biocontrol Science and Technology 20(6):567-587.

Wheeler, G.S., Pemberton, R.W., and Raz, L. 2007. A biological control feasibility study of the invasive weed – air potato, *Dioscorea bulbifera* L. (Dioscoraceae): an effort to increase biological control transparency and safety. Natural Areas Journal 27:269-279.

Chinese tallow biological control. Chinese tallow (*Triadica sebifera*, Euphorbiaceae) is an aggressive woody invader of wetland, natural, and disturbed coastal habitats in the United States. This weed originated from central-southern China and presently invades coastal areas from North Carolina, west to eastern Texas. Current control methods are limited to unsustainable and cost-prohibitive mechanical and herbicidal methods. ARS scientists in Fort Lauderdale, Florida, discovered and tested herbivorous species native to China for biological control of the weed. These include a leaf rolling weevil (*Heterapoderopsis bicallosicollis*), a flea beetle (*Bikasha collaris*), a caterpillar (*Gadirtha inexacta*), and an un-named fly gall former. Based on host-testing, *H. bicallosicollis* was found to be unsuitable and potentially dangerous. *B. collaris and G. inexacta* were suitable, exhibiting a narrow host range restricted to the target weed and a few other related plants from their native China. These two agents are now being developed for biological control.

Wang, Y., Zhu L., Gu, X., Wheeler, G.S., Purcell, M., and Ding, J. 2012. Pre-release assessment of a noctuid *Gadirtha inexacta* (=*Iscadia inexacta*) proposed as a biological control agent of Chinese tallow (*Triadica sebifera*) in the United States. Biological Control 63:304-309.

Huang, W., Wheeler, G.S., Purcell, M.F., and Ding, H. 2011. The host range and impact of *Bikasha collaris* (Coleoptera: Chrysomelidae), a promising candidate agent for biological control of Chinese tallow, *Triadica sebifera* (Euphorbiaceae) in the United States. Biological Control 56:230-238.

Wang, Y., Ding, J., Wheeler, G.S., Purcell, M.F., and Zhang, G. 2009**.** *Heterapoderopsis bicallosicollis* (Coleoptera: Attelabidae): A potential biological control agent for *Triadica sebifera*. Environmental Entomologist 38:1135-1144.

Biological control of the tropical soda apple. The tropical soda apple (*Solanum viarum)* is a weed native to South America that was accidently introduced into Florida and has become a serious problem in pastures and natural areas. Cattle avoid the spiny leaves and stems of the tropical soda apple, which gives it a chance to become dominant in a pasture. To control the weed, ARS explorations in Argentina identified a leaf-feeding beetle (*Gratiana boliviana*) that only survives on tropical soda apple. ARS scientists in Gainesville (and the Tallahassee worksite), Florida, collaborated with researchers from the University of Florida, the Florida Division of Plant Industry, APHIS, and the ARS South American Biological Control Laboratory in Argentina to implement a program to control tropical soda apple using the beetle. Beetles were mass reared in Florida and more than 250,000 adults were released between 2003 and 2011. Reductions of tropical soda apple densities due to feeding damage by the beetle were visible 2 to 3 years after initial release, and in some cases, within a few months. Various methods of technology transfer were used to inform the public, land owners, funding agencies, and scientists about the biological control program, including articles in trade magazines, extension publications, Web sites, videos, field days, and scientific publications. The tropical soda apple research team was acknowledged by the Florida Entomological Society with their "Achievement Award for Team Research" in 2010.

Manrique, V., Diaz, R., Hight, S.D., and Overholt, W.A. 2011. Evaluation of mortality factors using life table analysis of *Gratiana boliviana*, a biological control agent of tropical soda apple in Florida. Biological Control 59:354-360.

Medal, J., Bustamante, N., Overholt, W., Diaz, R., Stansly, P. Roda, A., Amalini, D., Hibbard, K., Gaskalla, R., Sellers, B., Hight, S., and Cuda, J. 2010. Biological control of tropical soda apple (Solanaceae) in Florida: Post-release evaluation. Florida Entomologist 93:130-132.

Overholt, W.A., Diaz, R., Hibbard, K.L., Roda, A.L., Amalin, D., Fox, A.J., Hight, S.D., Medal, J.C., Stansly, P.A., Carlisle, B., Walter, J.H., Hogue, P.J., Gary, L.A., Wiggins, L.F., Kirby, C.L., and Crawford, S.C. 2009. Releases, distribution and abundance of *Gratiana boliviana* (Coleoptera: Chrysomelidae), a biological control agent of tropical soda apple (*Solanum viarum*, Solanaceae) in Florida. Florida Entomologist 92:450-457.

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COMPONENT IV:
Protection of Post-Harvest Commodities and Quarantine

The problem of losses due to insect pests does not end with harvest. The value of raw commodities can be reduced by direct insect damage, and exotic insect pests must be detected and eliminated to ensure the safe movement of agricultural commodities from infested to non-infested areas through marketing channels. In addition, commodities such as grains (corn, wheat, and rice), nuts, and fruits are processed into value-added products that are susceptible to insect attack. Research outcomes from this component directly contribute to the development of effective and sound management strategies to reduce pest damage in post-harvest commodities so as to limit the spread of exotic pests within the United States and ensure U.S. competitiveness in the international commerce of agricultural commodities. Accomplishments also contribute to basic biology through their contributions to methods for genetic manipulation.

The NP 304 Action Plan includes two Problem Statements that were expected to guide the 5-year research plan and the development of the anticipated products in this Component. The Problem Statements and the research accomplishments that address each of them are presented below.

PROBLEM STATEMENT IVA: *Insect Pests of Fresh Commodities*

Fresh commodities such as grapes and strawberries are important trade items. Export of fresh commodities is an important part of the agricultural economy of the United States. Imported fresh commodities provide nutrition and enjoyment for Americans, especially when those food items are out of season in our own country. One of the challenges presented by this trade is the danger of moving invasive crop pests to places where they do not yet occur. Experience and scientific study have identified pests associated with each commodity. Systematic tests are required to establish methods for completely eliminating those pests from particular commodities, with special attention to protecting product quality. Methods cover a wide range of techniques, from broadly effective fumigation of entire shipments to careful detection and spot treatment. If an invasive pest is introduced, other strategies can be used to eliminate it before it can become established. The goal of these treatments is different from much of pest control in that the target pest must be completely eliminated, not just managed. From a research standpoint, these complicated requirements make it necessary to study a wide range of subjects, including basic biology of the pests, sensitive methods for detecting the pests, and robust control techniques.

The research under this Problem Statement was directed at some of the highest-priority pests that present continuing challenges to trade and production. The program is not large enough to treat all the pests of concern, but it has made major contributions toward control of the pests on which it concentrated. Those contributions included development of techniques with long-term potential in genetic control and products that should be useful in the near term.

***Systems for gene targeting and producing stable genomic transgene insertions*.** Limitations to the fundamental and practical application of transgenic insect strains, due primarily to the random nature of genomic vector insertions, can lead to mutations and variable transgene

expression and, secondarily, to the inherent instability of the (transposon-based) vector systems that are used to create them. These factors make the development of transgenic strains for applied use laborious because of the need to transform many individuals in order to get a useful transgenic line. The lack of targeted insertion also makes comparison of transgene function unreliable because the location of an insertion can affect function. ARS scientists in Gainesville, Florida, addressed these important issues by developing new genomic targeting systems based on recombinase-mediated cassette exchange (RMCE) that avoid genomic insertion sites subject to mutations and position effects. RMCE can then be used to modify an initial vector insertion so that any transposase present cannot re-mobilize the vector insertion, thus preventing strain instability and lateral transmission of transgenes into new species. These new vector transformation and modification systems are expected to enhance the development of transgenic strains for biologically based insect population control and enhance the environmental safety of field applications. For the sterile insect technique, genetic modification can create sex-linked, conditional lethal strains that produce only males when chemically stimulated. Such a strain would save mass rearing costs and also provide an extra level of safety, in that the insects released would have a genetic barrier against establishment. Directed genetic modification would also make other manipulations possible, such as strains that drive genes into wild populations for the purpose of eliminating negative characteristics of the pests. For example, a gene might be driven into the population that eliminates the threat of a vector or that modifies host preference of a species.

Schetelig, M.F. and Handler, A.M. 2013. A Functional comparison of the 3xP3 promoter by recombinase-mediated cassette exchange in Drosophila and a tephritid fly, Anastrepha suspense. G3 Genes Genomes Genetics 3:687-693.

Horn, C. and Handler, A.M. 2010. System for gene targeting and producing stable genomic transgene insertions. U.S. Patent and Trademark Office, Patent no. 7,700,356.

Schetelig, M.F., Scolari, F., Handler, A.M., Kittelmann, S., Gasperi, G., and Wimmer, E.A. 2009. Site-specific recombination for the modification of transgenic strains of the Mediterranean fruit fly, *Ceratitis capitata*. Proceedings of the National Academy of Sciences of the United States 106:18171-18176.

Transgenic tephritid fruit fly strains for the sterile insect technique. The sterile insect technique (SIT) is based on the principle of artificially releasing sterile males into wild populations of a pest to suppress fertility. SIT has been effective for suppression and even eradication of some important pest species, especially in the order Diptera. Effective SIT requires the production of millions or even billions of sterile males, an expensive and technically demanding effort. Any improvement in the efficiency of that rearing process can save significant funds. The strategy of inserting genes into the target species that allow survival of both sexes under special conditions but that prevent survival of females under other conditions offers the chance of much greater efficiency. Fruit flies (Tephritidae) include a number of species that attack fruit before harvest, sometimes completely ruining a crop. A number of those species have been kept out of the United States by strict quarantine procedures and response to spot infestations with insecticides and sterile male releases. Recently, the spotted wing drosophila, *Drosophila suzukii*, was introduced into the United States from Asia. This insect behaves similarly to a tephritid fly, though it is in the family Drosophilidae. To develop transgenic fruit fly strains that allow for separation of the sexes during mass rearing and

eliminate the need to use sterilizing radiation, ARS scientists in Gainesville, Florida, constructed "tetracycline-suppressible embryonic lethality strains" of the Caribbean fruit fly, *Anastrepha suspensa*. Two transgenic embryonic sexing strains exhibited total female-specific embryonic lethality, even when reared under large-scale conditions. This technology is now being developed for other important insect pests, including other tephritid species such as the Mexican fruit fly and the spotted-wing drosophila. It is expected that use of transgenic embryonic and female-specific conditional-lethal strains will greatly improve the efficacy and cost-effectiveness of population control measures by producing only males, which could lead to using less or no radiation in SIT breeding operations. New strain development, and eventual field tests, will be accomplished with collaborators at the FAO/IAEA Insect Pest Control Laboratory (Seibersdorf, Austria), Moscafrut (Tapachula, Mexico), and APHIS-PPQ.

Schetelig, M.F. and Handler, A.M. 2012. A strategy for enhanced transgenic strain development for embryonic conditional lethality in *Anastrepha suspensa*. Proceedings of the National Academy of Sciences of the United States 109:9348-9353.

Schetelig, M.F. and Handler, A.M. 2012. A transgenic embryonic sexing system for *Anastrepha suspensa* (Diptera: Tephritidae). Insect Biochemistry and Molecular Biology 42:790-795.

Schetelig, M.F., Nirmala, X., and Handler, A.M. 2011. Pro-apoptotic cell death genes, hid and reaper, from the tephritid pest species, *Anastrepha suspensa*. Apoptosis 16:759-768.

Diet and juvenile hormone interactions in the fruit fly SIT. SIT depends on the ability of intentionally released, sexually sterilized males to effectively compete for mates with normal, naturally occurring males. ARS scientists in Gainesville, Florida, studied the sexual behavior of Caribbean fruit flies, a pest that re-emerged in Florida in 1964 and is considered a potential commercial pest of citrus, mangoes, and peaches. Caribbean fruit flies were treated with a juvenile hormone analog, methoprene, and/or provided with a protein-enhanced diet. The scientists found that males provided with either methoprene or a protein-enhanced diet were up to twice as likely to exhibit traits associated with successful mating—initiation of mating aggregations, occupation of favorable locations within mating aggregations, performance of sexual signaling (chemical, visual, and acoustic), defeat of rivals in territorial conflicts, and greater attractiveness to females. Each treatment individually more than quadrupled male sexual success; when the treatments were given together, the effects were additive. As a result, males given both a protein diet and treated with methoprene were about 10 times more likely to mate than untreated competitors. Protein itself enhanced male longevity and, unexpectedly, a methoprene treatment did not negatively affect life span or lower lipid levels. Initally, the techniques involved were incorporated into the mass-rearing procedures of the Mexico nation-wide fruit fly program (MOSCAFRUTA), and changes in adult diet were subsequently made in SIT facilities worldwide (Argentina, Australia, Austria, France, Mexico, and Portugal).

Pereira, R., Teal, P., Conway, H., Worley, J., and Sivinski, J. 2011. Influence of methoprene and dietary protein on maturation and sexual performance of sterile *Anastrepha ludens* (Diptera: Tephritidae). Journal of Applied Entomology 135(9):1-9.

Pereira, R., Sivinski, J., and Teal, P. 2010. Influence of a juvenile hormone analog and dietary protein on male *Anastrepha suspensa* (Diptera: Tephritidae) sexual success. Journal of Economic Entomology 103(1):40-46.

Pereira, R., Sivinski, J., Teal, P., and Brockmann, J. 2010. Enhancing male sexual success in a lekking fly (*Anastrepha suspensa* Diptera: Tephritidae) through a juvenile hormone analog has no effect on adult mortality. Journal of Insect Physiology 56:1552-1557.

Aluja, M., Ordano, M., Teal, P., Sivinski, J., Garcia-Medel, D., and Anzures-Dadda, A. 2009. Larval feeding substrate and species significantly influence the effect of a juvenile hormone analog on sexual development/performance in four tropical tephritid flies. Journal of Insect Physiology 55:231-242.

Pereira, R., Sivinski, J. and Teal, P. 2009. Influence of methoprene and dietary protein on male *Anastrepha suspensa* (Diptera: Tephritidae) mating aggregations. Journal of Insect Physiology 55:328-335.

Acoustic detection of hidden pest insects in trees and reductions in instrument costs. Acoustic technology is useful for the early detection of hidden invasive pest insects in trees, but its wide-spread utility requires that signal processing methods be able to discriminate between target insect sounds and background noise. ARS scientists in Gainesville, Florida, in collaboration with researchers in the Caribbean, Australia, Spain, and Saudi Arabia, identified temporal patterns of feeding and movement activity that help distinguish target pests' sounds from non-target sounds and background noise. Working with electrical engineers, these researchers developed microcomputer-controlled sensors and detection devices that are about10-fold lower in cost than commercially available instrumentation systems. As an indicator of demand, five companies have now expressed interest in buying these devices, and agricultural industry personnel are working with the ARS scientists using an instrument from Acoustic Emission Consulting, Inc. This success has led to greater interest in and acceptance of acoustic technology for insect detection and surveillance, particularly for managing invasive species, including the red palm weevil, Asian long-horned beetle, and bed bug, and stored-product insects such as the rice weevil, red flour beetle, and drugstore beetle.

Mankin, R.W., Hodges, R.D., Nagle, H.T., Schal, C., Pereira, R.M. and Koehler, P.G. 2010. Acoustic indicators for targeted detection of stored product and urban insect pests by inexpensive infrared, acoustic, and vibrational detection of movement. Journal of Economic Entomology 103:1636-1646.

Mankin, R.W., Mizrach, A., Hetzroni, A., Levsky, S., Nakache, Y., and Soroker, V. 2008. Temporal and spectral features of sounds of wood-boring beetle larvae: identifiable patterns of activity enable improved discrimination from background noise. Florida Entomologist 91:241-248.

Mankin, R.W., Smith, M.T., Tropp, J.M., Atkinson, E.B., and Jong, D.Y. 2008. Detection of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) larvae in different host trees and tissues by automated analyses of sound-impulse frequency and temporal patterns. Journal of Economic Entomology 101:838-849.

Bait station for control of tephritid fruit flies. Fruit flies (Tephritidae) include a number of species that attack fruit before harvest, sometimes completely ruining a crop. A number of those species have been kept out of the United States by strict quarantine procedures and response to spot infestations with insecticides and sterile male releases. ARS scientists in Miami, Florida, developed a wax-based matrix that contains an attractant, a feeding stimulant, and a visual cue that provides a long-lasting delivery system for control of pest fruit flies while using very little pesticide per acre. ARS has filed a U.S. patent application that describes a bait station that uses

inexpensive materials (such as cardboard) as its support/holder device, is coated with a toxicant-infused wax matrix, and is used with a long-lasting fruit fly lure. Under a formal agreement with industry, the bait stations are being commercialized using spinosyn insecticide. The product will control Mediterranean, Caribbean, Mexican, and West Indian fruit flies. This will provide environmentally friendly, commercially available alternatives for use by regulatory agencies as well as by growers.

Epsky, N.D., Midgarden, D., Rendón, P., Villatoro, D., and Heath, R.R. 2012. Efficacy of wax matrix bait stations for Mediterranean fruit flies (Diptera: Tephritidae). Journal of Economic Entomology 105(2):471-479.

Heath, R.R., Rendón, P., and Epsky, N.D. 2012. Insect bait station method and apparatus. U.S. Patent and Trademark Office, Patent Application no. 13/397,799; filed February 2012.

Identification of attractants for detection of the redbay ambrosia beetle. Redbay ambrosia beetle is an invasive wood-boring beetle that vectors the fungal pathogen that causes laurel wilt, a lethal disease threatening the avocado industry. An effective lure for early detection of the redbay ambrosia beetle is a critical need for growers and action agencies. ARS scientists in Miami, Florida, documented that the current redbay ambrosia beetle lure (manuka oil) has a field life of only 2–3 weeks in Florida due to rapid loss of attractive sesquiterpenes, primarily α-copaene. The scientists developed new methods to accelerate identification of beetle attractants, including quantification of sesquiterpene emissions using analytical chemistry, collection of host-seeking beetles in the field, and direct measurement of olfactory response from redbay ambrosia beetle antennae using electrophysiology techniques. Based on the results from laboratory studies, seven plant essential oils were evaluated in field tests in 2012, and cubeb oil (a distillate from berries of *Piper cubeba*) was identified as a new attractant for redbay ambrosia beetle. ARS is currently preparing a cooperative agreement with an industry partner to develop improved redbay ambrosia beetle lures based on cubeb oil.

Kendra, P.E., Montgomery, W.S., Niogret, J., and Epsky, N.D. 2013. An uncertain future for American Lauraceae: A lethal threat from redbay ambrosia beetle and laurel wilt disease. American Journal of Plant Sciences 4(3A):727-738.

Kendra, P.E., Niogret, J., Montgomery, W.S., Sanchez, J.S., Deyrup, M.A., Pruett, G.E., Ploetz, R.C., Epsky, N.D., and Heath, R.R. 2012. Temporal analysis of sesquiterpene emissions from manuka and phoebe oil lures and efficacy for attraction of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). Journal of Economic Entomology 105(2):659-669.

Kendra, P.E., Montgomery, W.S., Sanchez, J.S., Deyrup, M.A., Niogret, J., and Epsky, N.D. 2012. Method for collection of live redbay ambrosia beetles, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). Florida Entomologist 95(2):513-516.

Kendra, P.E., Montgomery, W.S., Niogret, J., Deyrup, M.A., Guillén, L., and Epsky, N.D. 2012. *Xyleborus glabratus, X. affinis,* and *X. ferrugineus* (Coleoptera: Curculionidae: Scolytinae): Electroantennogram responses to host-based attractants and temporal patterns in host-seeking flight. Environmental Entomology 41(6):1597-1605.

PROBLEM STATEMENT IVB: *Insect Pests of Durable (Stored and Processed) Commodities*

Insect damage to value-added products such as stored grain and prepared foods is the most expensive kind of damage for manufacturers. Although damage to the U.S. grain supply is thought to be only around 10 percent, the effect can be devastating for an individual facility, and a product can be completely ruined for the consumer. Insecticides are a valuable tool for integrated management of insect pests of stored and processed products, but they are limited by their ability to reach insects, many of which are in direct contact with foods. Methyl bromide had been used extensively to treat exports and for grain storage and for spot treatment in milling facilities, but its use has been extremely limited by international treaty due to its damaging effects on Earth's ozone layer. Other fumigants, such as sulfuryl fluoride, are not as effective and have limitations on use, and they are coming under increasing scrutiny from regulatory authorities. Considering the value of stored products and the extreme challenge presented by pests, it is imperative that consistent, innovative research for the development of entirely new ways of managing these arthropods be promoted.

ARS research under this Problem Statement produced new methods for treating export commodities, including grapes, hay, and beans. The procedures protected $1 billion in exports and reduced the need for harmful methyl bromide fumigation. The program also performed significant basic work in genetics. Using the red flour beetle, researchers sequenced the entire genome, found new ways to direct mutagenesis, and developed a gene drive mechanism. These discoveries have implications not only for protection of commodities, but for biology in general.

Methyl bromide alternative for insect control for export of table grapes and sweet cherries. Spotted wing drosophila (*Drosophila suzukii*) is a newly found invasive pest in the western United States that threatens the ability of growers to export California-grown table grapes and sweet cherries—which have an annual export value estimated at $200 million—to Australia and New Zealand. ARS scientists in Parlier, California, developed a combination of sulfur dioxide fumigation and cold treatment as a postharvest alternative to methyl bromide fumigation for controlling this pest in California-grown table grapes. The scientists further enhanced the ability of producers to export grapes by developing a method to remove fungicide residues using ozone fumigation. This work has provided stakeholders with market access to Australia and New Zealand. Following requests of the western U.S. cherry industry, the scientists also completed the validation of a quarantine treatment utilizing methyl bromide fumigation. This research enabled the retention and expansion of market access to Australia, estimated at $55 million annually.

Bellamy, D.E., Hall IV, W.A., Sisterson, M.S., and Walse, S.S. 2013. The development of a host potential index and its postharvest application to the spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). PLoS One 8(4):e61227.

Johnson, J.A., Walse, S.S., and Gerik, J.S. 2012. Status of alternatives for methyl bromide in the United States. Outlooks on Pest Management 23(2):53-58.

Walse, S.S., Krugner, R., and Tebbets, J.S. 2012. Postharvest treatment of strawberries with methyl bromide to control spotted wing drosophila, *Drosophila suzukii*. Journal of Asia-Pacific Entomology 15:451-456.

Walse, S.S., Karaca, H., and Smilanick, J.L. 2011. Fungicide residue remediation on table grapes using ozone fumigation. Environmental Science and Technology 45(6):6961-6969.

New quarantine treatment for hay exports to Japan. Quarantine strategies are needed to control Hessian fly, a pest of regulatory concern in hay exported to Asia. ARS scientists in Parlier, California, developed a multi-step quarantine treatment using bale compression in modern compressors that produce various styles of export-quality bales and combined it with shortened 3-day hydrogen phosphide fumigation. This new treatment was evaluated in a large-scale commercial test to confirm complete control of Hessian fly in hay exported to Japan. Approval of the new certified quarantine treatment would assure trade partners that the insect would not be accidentally introduced through hay exported from the western states. The treatment would expedite shipments of high-quality U.S. hay exports to Japan and help alleviate feed shortages. Western U.S. hay exports to Asian countries are valued at $675 to $750 million, with additional revenues of $100 million annually for the ocean freight industry.

Yokoyama, V.Y. and Cambron, S.E. 2013. Survival of Hessian fly (Diptera: Cecidomyiidae) puparia after exposure to simulated hay harvest conditions, drying on location and in windrows in Washington and California. Journal of Economic Entomology 106(3):1164-72.

Yokoyama, V.Y. 2012. The Japan disaster and U.S. hay exports. American Entomologist 58:151-153.

Yokoyama, V.Y. 2011. Approved quarantine treatment for Hessian fly (Diptera: Cecidomyiidae) in large-size hay bales and Hessian fly and cereal leaf beetle (Coleoptera: Chrysomelidae) control by bale compression. Journal of Economic Entomology 104:792-798.

Control of Indianmeal moth with mating disruption. Indianmeal moth is the most important moth pest of stored products worldwide, feeding on a wide variety of stored products, including herbs, nuts, grains, and other pantry items. An infestation quickly ruins the entire product, in part because the larvae leave webs wherever they go. The use of sex pheromones to interfere with the reproductive cycle of pests (mating disruption) is safer and less disruptive to the operations of commercial food processing facilities than fumigants and aerosol sprays (insecticides), which commonly require multiple applications. One impediment to the adoption of mating disruption is the potential interference with use of pheromone traps for monitoring Indianmeal moth populations. ARS scientists in Parlier, California, discovered that the moth continues to enter monitoring traps even during the use of pheromones for mating disruption. These findings provide assurances to applicators of mating disruption techniques that they still will be able to monitor the results of their efforts while the control is in progress.

Burks, C.S. and Kuenen, L.P. 2012. Effect of mating disruption and lure load on the number of *Plodia interpunctella* males captured in pheromone traps. Journal of Stored Products Research 49:189-195.

Burks, C.S., McLaughlin, J.R., Miller, J.R., and Brandl, D.G. 2011. Mating disruption for control of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) in dried beans. Journal of Stored Products Research 47:216-221.

Development of radio frequency treatments against the cowpea weevil. Cowpea weevils infest lentils, chickpeas, beans, and peas while the crops are in the field. Larvae continue to eat the bean while it is in storage, destroying infested beans and sometimes creating a mold problem for the entire storage unit. Infestations can cause entire shipments to be refused for export. Radio frequency heat treatments provide a non-chemical means of controlling cowpea weevils, but development of effective radio frequency control treatments requires the identification of protocols that are efficacious against the most heat-tolerant life stage of the pest. Such treatments must be adjusted so that they do not negatively affect product quality. ARS scientists in Parlier, California, working with collaborators at Washington State University, determined that the late larval and pupal stages are most heat tolerant and that short-term exposures to temperatures of 60°C, obtained through a combination of radio frequency heating and hot forced air, is an effective control that does not cause product damage. Additional studies by the scientists compared the dielectric properties of the pest and target commodities to improve treatments. These studies also showed that adult weevils are unlikely to escape treatment by leaving the product. This information will be used to develop fast, non-chemical radio frequency heat treatments that can reduce the need for fumigants while maintaining export markets worth $90 million. Industry has shown interest in developing this method commercially for other commodities. The equipment is readily available for a variety of uses.

Jiao, S., Johnson, J.A., Tang, J., Wang, S. 2012. Industrial-scale radio frequency treatments for insect control in lentils. Journal of Stored Products Research 48:143-148.

Jiao, S., Tang, J., Johnson, J.A., Tiwari, G., and Wang, S. 2011. Determining radio frequency heating uniformity of mixed beans for disinfestation treatments. Transactions of the ASABE 54(5):1847-1855.

Jiao, S., Johnson, J.A., Tang, J., Tiwari, G., and Wang, S. 2011. Dielectric properties of cowpea weevil, black eyed peas and mung beans with respect to the development of radio frequency heat treatments. Biosystems Engineering 108(3):280-291.

Wang, S., Tiwari, G., Jiao, S., Johnson, J.A., and Tang, J. 2010. Developing postharvest disinfestation treatments of legumes using radio frequency energy. Biosystems Engineering 105:341-349.

Guo, W., Wang, S., Tiwari, G., Johnson, J.A., and Tang, J. 2010. Temperature and moisture dependent dielectric properties of legume flours associated with dielectric heating. Lebensmittel Wissenschaft und Technologie. Food Science and Technology 43:193-201.

Tribolium genome sequenced. The red flour beetle, *Tribolium castaneum*, is a major worldwide pest of stored grains in flour mills and household pantries. It can reproduce abundantly in grain and flour, ruining products due to contamination with insect parts and mold and simply through consumption. There are few good options for controlling this insect because removal of infested grain or flour costs millions in product losses, and direct treatment with certain pesticides could pose significant human risks. ARS researchers in Manhattan, Kansas, in collaboration with Kansas State University and Baylor College of Medicine researchers, sequenced the *T. castaneum* genome, the first beetle ever sequenced. This achievement has impact both as fundamental research and as a resource for finding vulnerabilities of the pest so that new control measures with different modes of action can be discovered.

Semeo, A.A., Campbell, J.F., Beeman, R.W., Lorenzen, M.D., Whitworth, R.J., and Sloderbeck, P.E. 2012. Genetic structure of *Tribolium castaneum* (Coleoptera: Tenebrionidae) populations in mills. Environmental Entomology 41:188-199.

Richards, S., Gibbs, R.A., Weinstock, G. M., Brown, S.J., Denell, R., Beeman, R.W., and Nguyen, N. 2008. The genome of the model beetle and pest *Tribolium castaneum*. Nature 452(7190):949-955.

Selfish gene discovered. *Tribolium castaneum,* the red flour beetle, and *Tribolium confusum,* the confused flour beetle, are similar tenebrionid beetles highly adapted to consumption of stored products. They can ruin entire silos of grain or flour and also appear anywhere in a flour mill where grain material accumulates. The flour beetles are also major pests in retail outlets and homes. Because of their importance, they have been one of the first insect targets of advanced genetic studies. The unique, "selfish" gene, *Medea* (*M*), is known to be widespread in natural populations of *Tribolium* beetles but is unknown in the rest of the invertebrate world. Heterozygous *Medea* females (M/+) transmit a dominant lethal gene to hatchlings, but the lethal effect is manifested only in those progeny that do not inherit an *M* allele from either parent. Thus, each *M* allele is bi-functional, encoding both a maternally loaded "poison" and an "antidote" expressed in the embryo. These properties favor the transmission of the *M* allele to subsequent generations at the expense of the non-*M* allele and account for their selfish behavior. ARS researchers in Manhattan, Kansas, used recombination mapping and sequence comparison (wild-type genome vs. M^1-derived sequence) to discover a 21.5-kb DNA insertion at one far end of chromosome 3 in M^1 beetles. The researchers also demonstrated that approximately 50 percent of all *T. castaneum* populations sampled worldwide carry the M^1 gene. ARS researchers mapped M^4, finding it on the end of chromosome 3 (opposite M^1). It is possible that genetic manipulation of *M* genes could drive specific genetic traits rapidly into a population and significantly diminish the beetles' survival and spread. Use of *Medea* genes in other insects such as vectors of malaria and dengue has been proposed and, if successful, would have major impacts on controlling these devastating diseases. These basic discoveries in genetic physiology have influenced thinking about organismic biology and have provided concepts for entirely new methods of insect genetic control.

Trauner, J., Schinko, J., Lorenzen, M.D., Shippy, T.D., Wimmer, E.A., Beeman, R.W., Klingler, M., Bucher, G. and Brown, S.J. 2009. Large-scale insertional mutagenesis of a coleopteran stored grain pest, the red flour beetle *Tribolium castaneum*, identifies embryonic lethal mutations and enhancer traps. BMC Biology 7:73.

Lorenzen, M.D., Gnirke, A., Margolis, J., Campbell, M., Stuart, J.J., Aggarwal, A., Richards, S., Park, Y. and Beeman, R.W. 2008. The maternal-effect, selfish genetic element *Medea* is associated with a Tc1 transposon. Proceedings of the National Academy of Science of the United States 105:10085–10089.

New genomic tools developed. One of the practical results from basic genomic studies that is beginning to be appreciated is the use of small segments of RNA that inhibit production of specific proteins. Typically consisting of short segments of double-stranded RNA (dsRNA), the interfering RNA goes through a series of steps collectively called RNA interference (RNAi). One of the key steps is recognition of a specific gene sequence by the opposing RNA sequence originally on the dsRNA. This is a natural process used widely among organism for, among other functions, resisting viruses. However, it is possible to manipulate the process by inserting

dsRNA sequences that intentionally inhibit proteins used by the organism itself. This provides a tool to study the function of genes and enzymes, but it also is being examined closely as a source of insecticides composed of RNA. The advantages to such an insecticide would be great specificity, even to the species level; non-persistence in the environment; and total safety for the applicator and consumer. ARS scientists in Manhattan, Kansas, worked with the red flour beetle, *Tribolium castaneum*, to show that injection of dsRNA into that species created a systemic effect in which the dsRNA reproduced itself throughout the beetle. The researchers used this technique to target genes with unknown function, observing the effect on the beetles. In this way, they were able to identify components of exoskeleton biosynthesis and degradation pathways. The scientists have functionally characterized a wide range of chitin/cuticle pathway genes, revealing a wealth of potential targets for arthropod-specific pest control. The ARS researchers were also instrumental in the development and use of a transposon-based system for genome-wide mutagenesis in *Tribolium*, which resulted in new genome insertions, some of which interrupt normal gene function. This system was used to generate more than 15,000 new insertions. This approach has several advantages over chemical mutagenesis because its mutations are the direct result of transposon insertion, creating a recognizable "tag" on the inserted gene. The insertion can therefore be quickly identified using standard molecular techniques such as inverse or universal polymerase chain reaction. Transposon-based mutagenesis is a complementary method to the RNAi-based approach, as the former does not require knowledge of the target gene and the latter provides the means to select a gene for study from among those with known sequences. The development of these useful molecular techniques will lead to a better understanding of the physiology and adaptability of *Tribolium* pests, improving the chances of more effective, safer control. Major pesticide manufacturers are developing products based on RNAi, building on the foundation discovered by ARS.

Broehan, G., Arakane, Y, Beeman, R.W., Kramer, KJ, Muthukrishnan, S., and Merzendorfer, H. 2010. Chymotrypsin-like peptidases from *Tribolium castaneum*: a role in molting revealed by RNA interference. Insect Biochemistry and Molecular Biology 40(3):274–283.

Trauner, J., Schinko, J., Lorenzen, M.D., Shippy, T.D., Wimmer, E.A., Beeman, R.W., Klingler, M., Bucher, G., and Brown, S.J. 2009. Large-scale insertional mutagenesis of a coleopteran stored grain pest, the red flour beetle *Tribolium castaneum*, identifies embryonic lethal mutations and enhancer traps. BMC Biology 7:73.