National Program 304
Crop Protection and Quarantine
FY 2012 Annual Report

In memoriam

John Lydon, Ph.D., died on October 19, 2012, from injuries received in a car accident during his visit to the ARS laboratory in Parlier, California. John shared responsibility for National Program 304 (Plant Protection and Quarantine) with Kevin Hackett and Daniel Strickman. He was an exceptional national program leader and friend who made invaluable contributions to this program. He will be greatly missed.

Introduction

The Crop Protection and Quarantine National Program (NP 304) addresses high priority insect, mite, and weed pest problems of crops, forests, urban trees, rangelands, postharvest systems (such as stored grains), and natural areas. The goals of NP 304 are twofold: to understand the biology, ecology, and impact of these pests on agricultural production and natural systems and to develop, improve, and integrate environmentally safe technologies to exclude, eradicate, or manage pest populations. Priority is placed on sustainable and integrated practices that enhance the productivity, quality, and safety of U.S. agriculture while protecting natural resources, native ecosystems, human health, and the environment.

This National Program is divided into four research components:

- *Systematics and Identification*: accurately identifying insects, mites, and weeds, whether native or invasive, to get important information about their possible country of origin and bionomics and the taxonomy and systematics of microorganisms associated with these insects and weeds for aid in developing microbials as biological control agents
- *Protection of Agricultural and Horticultural Crops*: improving existing and/or developing new, innovative control strategies for pests in traditional and organic agricultural and horticultural systems
- *Protection of Natural Ecosystems*: preventing, managing, and controlling critical insect pests and weeds that threaten environmental areas and the agricultural areas bordering them
- *Protection of Postharvest Commodities and Quarantine*: contributing to the development of effective and sound management strategies to reduce pest damage in postharvest commodities, limit the spread of exotic pests within the United States, and ensure U.S. competitiveness in the international commerce of agricultural commodities

Below are research accomplishments for this national program from fiscal year 2012. The results are presented under the components and problem areas of this program's 2010-2015 Action Plan. The report below is not intended to be a progress report describing all research conducted during the 2012 fiscal year, rather it is an overview that highlights major accomplishments, some of which are based on multiple years of research.

ARS welcomes your input regarding our ongoing research programs. If you have any questions, please do not hesitate to contact the co-leaders of National Program 304, Kevin Hackett (Kevin.Hackett@ars.usda.gov) and Dan Strickman (Daniel.Strickman@ars.usda.gov).

Component 1: Systematics and Identification

PROBLEM STATEMENT 1A: INSECTS AND MITES

Systematics of the emerald ash borer and related species. The emerald ash borer, *Agrilus planipennis*, is a very significant invasive beetle killing ash trees in the United States. ARS researchers in Beltsville, Maryland, along with Forest Service collaborators in East Lansing, Michigan, and a collaborator in St. Petersburg, Russia, developed a morphology-based taxonomy for identification of the immature stages of the emerald ash borer. Prior to this study, the immature life stages were poorly known and had never been fully described and illustrated. This morphology-based taxonomy will be a valuable tool for the rapid identification of this serious invasive pest beetle in the United States.

New genus and species of parasitic wasp and the first report of a true myrmecophilic (ant-loving) wasp species. Parasitic wasps help control populations of pest ants and have been evaluated for potential use as biological control agents of ants. Certain tropical ants form nests in the canopies of citrus and coffee trees and often contribute to leaf loss and protect plant-feeding insects that infest these crops. This can lead to losses in crop productivity costing millions of dollars annually. A new genus and species was taxonomically described, illustrated, and its host use was reported. Character diagnostics were discussed in the context of other species and groups of species in the same family and relative to more distantly related myrmecophilic (ant-loving) wasps. This research is useful to other scientists conducting research on myrmecophilic wasps and their hosts as well as pest management and regulatory personnel responsible for controlling and limiting the spread of pest ants.

Mite and insect identification services. The Taxonomic Support Unit (TSU) of the ARS Systematic Entomology Laboratory in Beltsville, Maryland, provides identifications and information on important mite and insect invasive and adventive species. Requests for support come from many sources including the Animal and Plant Health Inspection Service (APHIS), State Departments of Agriculture, and U.S. universities. At the time of reporting on October 1, 2012, the mite section identified 387 lots with 2,395 mite specimens. For FY2012, the TSU identified 9,892 lots with 25,015 arthropod (insect and mite) specimens. It is reasonable to assume that the rate of identifications will remain constant for the rest of the 2012 fiscal year. If so, these numbers represent 75 percent of the expected total for the 2012 calendar year. These identifications are an essential to successful protection of our borders from invasion by mites and insects.

PROBLEM STATEMENT 1B: WEEDS

Root physiology for weed control and plant health. Herbicides are an essential part of American agriculture, not only to increase yields of major crops, but also to preserve soil and water through no-till strategies. Herbicide resistance threatens the usefulness of active ingredients, some of which are less damaging to the environment. Basic work on the physiology of root hairs by ARS scientists in Oxford, Mississippi revealed an entirely new mechanism of plant physiology that might be exploited for the development of

herbicides. Using a library of the DNA sequences of root hair cells from sorghum, these scientists identified the genetic sequences responsible for production of a natural herbicide, sorgoleone, and for the control of that gene. DNA with those sequences inserted into rice or mustard stimulated production of sorgoleone, which is normally only produced by sorghum. This discovery could lead to the development of crops that protect themselves from weeds, modify the microbial community in soil, enhance plant nutrient acquisition, and bioremediate contaminated soils.

PROBLEM STATEMENT 1C: MICROBIALS

Genetic clarification and reclassification of fungi in the Entomophthorales order. Fungi in the Entomophthorales order include some of the most important worldwide insect pathogens, but the relationships among these fungi and with other fungal groups have remained comparatively unstudied until now. Multigenic studies have proven that this group of fungi is a closely related taxonomic unit and supported the previous, traditionally based, classification into six families. However, several of the genera in these six families will need further study because the existing relationships among them were not supported by these studies. Several distinct lineages were recognized, and this year, ARS researchers in Ithaca, New York, reclassified Entomophthorales as a new fungal phylum named, Entomophthoromycota, which is comprised of three newly recognized classes (Basidiobolomycetes, Neozygitomycetes, and Entomophthoromycetes). This information will improve efforts to employ these fungi in biological control of insects.

Refuting role of destruxins' importance in insect pathogenicity and virulence by Metarhizium fungi. The small peptide family known as destruxins has been thought to be critically important for virulence against insects, but conclusive proof as to their importance has been lacking. ARS researchers in Ithaca, New York, identified the gene cluster responsible for biosynthesis of these peptides and generated knockout fungal strains of *Metarhizium* incapable of producing destruxins. The mutants had no significant changes in virulence levels against several different insect species and no changes in fungal morphology or development. Gene expression was detectable at low levels during early growth phase and increased with culture time; gene transcripts were also detectable in later stages of infected insects and in conidia. That destruxins appear to be dispensable for virulence against insects casts serious doubt on their long-purported role in insect pathogenicity and virulence. This information will inform efforts to tailor these fungi more specifically to insect targets.

Component 2: Protection of Agricultural and Horticultural Crops

PROBLEM STATEMENT 2A: BIOLOGY AND ECOLOGY OF PESTS AND NATURAL ENEMIES

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 scientists at Ohio State University, 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, practical, and environmentally friendly strategy to control pest insects by disrupting diapause.

Effects of global change on insect pests of crops. Global change may include an overall increase in global temperatures, redistribution of rainfall patterns with overall increase in precipitation, increased incidence of severe weather events, and increased levels of ambient carbon dioxide in the atmosphere. Agriculture must adapt to these environmental changes while increasing its total yields to meet the demand of an increasing world population. ARS researchers in Sidney, Montana, summarized the literature on climate change effects on insect pests for the National Climate Assessment for Agriculture. Rising air temperatures affect all aspects of insect life cycles and generally result in larger insect populations, resulting from earlier emergence and more generations per year. Many pests currently limited by cooler temperatures at higher latitudes will be able to expand their ranges into these areas. Elevated carbon dioxide levels cause plants to shift their defenses from the production of defensive chemicals to the development of tougher, less digestible leaves. Beetles, aphids, and possibly other pests are favored by these changes. Plans to grow more suitable crops as conditions change will need to consider the redistribution of pests and their increasing severity. Better integrated pest management can be developed for those pests likely to cause the greatest losses in yield. At this time, these conclusions are being disseminated in a status report on the impacts of climate on agriculture in the United States for the U.S. Congress and the President of the United States.

Localization of bacterial and fungal pathogens within the southern green stink bug. Southern green stink bugs (SGSBs) acquire several opportunistic pathogens from environmental sources as they feed. 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 transmitted to cotton bolls by SGSBs that are responsible for boll rot. These researchers determined that only two of the many pathogens found in the mouthparts, alimentary canal, or head of the SGSB, are actually transmitted into cotton bolls through SGSB feeding. This work provides a much better understanding of the role of the SGSB in transmitting pathogens and will be used to develop control measures to improve U.S. cotton yields.

Monitoring Ambrosia beetle in ornamental tree nurseries. *Xylosandrus crassiusculus* and *X. germanus* are the most problematic ambrosia beetles in ornamental nurseries in the Northeastern, Midwestern, Atlantic, and Southeastern states. ARS scientists in Wooster, Ohio, determined that ethanol-injected trees can be used to monitor ambrosia beetle flight and attack activity in nurseries; such trees were found to be more attractive to ambrosia beetle than trees drenched with ethanol or traps baited with ethanol. This will help growers synchronize their control treatments with ambrosia beetle activity and improve management of these pests.

New lures for critical pests. The spotted wing drosophila (SWD) is an invasive pest of soft fruits. The brown marmorated stink bug (BMSB) is a pest of many fruits, vegetables, and field crops. Both originated in Asia and are spreading throughout North America. ARS researchers in Wapato, Washington, working with ARS colleagues in Poplarville, Mississippi, and Oregon State Department of Agriculture scientists, isolated and identified a set of chemicals from the odors of wine and vinegar that can be used as a lure for SWD. ARS researchers in Beltsville, Maryland, and Kearneysville, West Virginia, discovered a male-produced pheromone that causes BMSB nymphs and adults to aggregate. Both lures are being combined with traps and will be used to monitor pest populations for treatment and, perhaps, to control the insects via trapping or insecticidal baits. A provisional patent has been filed for the stink bug lure.

Stabilization of beneficial traits enhances effectiveness of biological control. Biological control (the use of predators, parasitoids, or pathogens in pest suppression) provides a safe alternative to the use of chemical insecticides. However, during mass production, biological control agents can lose some beneficial traits, such as virulence and reproductive capacity, which can make the agents less effective in pest suppression. ARS researchers in Byron, Georgia, and colleagues at Brigham Young and Rutgers universities, discovered that beneficial trait loss can be prevented by selecting inbred lines. Inbred line technology has been adopted by three commercial companies that produce insect-killing nematodes.

PROBLEM STATEMENT 2B: CONTROL

Improvement of the sterile insect technique in fruit flies. ARS scientists in Tifton, Georgia, improved the efficacy of the Sterile Insect Technique (SIT) used to control tephritid fruit flies. They incorporated the juvenile hormone analog methoprene (which coordinates sexual signaling and reproductive development) and a protein supplement into the diets of adult sterile male flies. Flies fed the protein supplement became sexually mature 4–7 days earlier, thus mated earlier, and attracted more wild mates, thus increasing mating frequency. The International Atomic Energy Agency and the Food and Agricultural Organization are including this technology in a coordinated research program to improve the efficacy of SIT. The technology is now used in Mexico to improve the reproductive performance of sterile fruit flies that have been released to control invasive populations.

Lower cost equipment for sterilizing insect pests. The “Sterile Insect Technique” (SIT) is an effective method of biocontrol for insect pests worldwide, but SIT research is limited by both the high price (>$250,000) of commercially-available x-ray units and their poor performance in insect sterilization. In addition, gamma-based sources are increasingly unavailable due to heightened control of radioisotopes. ARS researchers in Albany, California, designed and built an economical and effective irradiation device for SIT applications. The effectiveness of this new design was validated by the sterilization of adult male navel orangeworm (pistachio pest). Material costs for the x-ray unit developed by ARS are less than $50,000 and a patent application has been filed. The work was partially funded by USDA, APHIS.

Plant growth bioregulators found to protect pecan foliage from black pecan aphids. Black pecan aphid feeding causes chlorosis (loss of photosynthetic chlorophyll pigment). ARS researchers in Byron, Georgia, found that treating pecan foliage with certain plant growth bioregulators lessens the chlorophyll degradation caused by the aphid, thus improving canopy health. Applying a plant growth bioregulator such as gibberellic acid also increases season-long photosynthesis by encouraging longer foliage retainment into the autumn. Return bloom is not affected and there appears to be little to no negative effect on beneficial insects. This new management tool is being adopted by pecan growers. Use of this bioregulator is also applicable to other crops with pests that elicit leaf chlorosis.

New genetic modifications yield "sexing strains" in fruit flies. Mass release of sterile, male fruit flies is used to control the insects, but sterile females released along with males do not contribute to fruit fly control and may distract the sterile males, thereby lowering control rates. Conditional-lethality, in which an insect's offspring dies when certain environmental conditions prevail, is a means of eliminating females early in their embryonic development. Females are not released and rearing costs are reduced. A conditional-lethal strain of the Caribbean fruit fly was created through genetic modification; females of this strain only survive when provided an antibiotic-(tetracycline) supplemented diet, therefore only male progeny are produced on an antibiotic-free diet. The genetic constructs involved in the production of conditional-lethal strains will serve to improve the efficacy of control programs that protect U.S. agriculture from fruit flies and other potentially invasive pests.

New methods using fungus to kill insect pests. Root weevils, soil grubs, rootworms, wireworms, fruit flies, and root maggots are insect pests that affect a wide variety of agricultural crops, landscape plants, and turf. ARS researchers in Peoria, Illinois, developed new methods to grow and commercially produce a bioinsecticidal fungus to kill these pests. The fungus, *Metarhizium*, also kills lesser meal worm larvae and adults, which are pests in commercial poultry operations. Koppert BV has licensed the technology from ARS through a patent. ARS researchers also developed dried granular formulations of *Metarhizium*, which will give homeowners, farmers, and land managers an effective, nonchemical way to control numerous soil-dwelling insect pests.

Protecting pecan foliage from black pecan aphids with plant growth bioregulators. Maintaining healthy pecan foliage is critical to the economic viability of producing and selling pecan nuts. ARS researchers at Byron, GA, found that treating pecan foliage with certain plant growth bioregulators (e.g., gibberellic acid) improves canopy health by lessening the negative impact of chlorophyll degradation from feeding by the black pecan aphid. Application of these plant growth regulators also increases season-long photosynthesis by retaining foliage longer into the autumn. Additionally, return bloom is not affected, and there appears to be little or no negative impact on beneficial insects. This new management tool is beginning to be adopted by growers and the approach is applicable to other crops experiencing pests that elicit leaf chlorosis through feeding.

Component 3: Protection of Natural Ecosystems

PROBLEM STATEMENT 3A: INSECTS

Managing emerald ash borer infestation. ARS researchers in Ithaca, New York, determined the extent of an emerald ash borer beetle infestation in western New York by using more than 100 individual (sentinel) girdled ash trees located up to 10 km away from the likely center of the infestation. Clusters of girdled ash trees were established near the center to act as sinks to attract potentially dispersing beetles. Trees within clusters were more heavily infested (nine larvae per square meter of bark) than sentinel trees or ungirdled trees. This indicates that clusters of girdled trap trees that are destroyed prior to the following season can help lower local densities of emerald ash borer, a finding that will be of interest to land managers.

Improved rearing methods for mass production of the introduced parasitic wasp T. planipennisi as a biocontrol agent of emerald ash borer. The emerald ash borer (EAB) is an invasive beetle pest that has killed millions of North American ash trees since its detection in 2002. The parasitic wasp *T. planipennisi* is an important biological control agent attacking EAB larvae. ARS researchers in Newark, Delaware, found that wasp offspring production, body size, and female to male ratio can be significantly enhanced by using healthy emerald ash borer larvae reared with tropical ash logs. The USDA, APHIS Emerald Ash Borer Biocontrol Laboratory (Brighton, MI) has applied this finding in the mass rearing of parasitic wasps for field releases against emerald ash borers in the United States, thus providing an important tool in the biological control effort against these devastating bugs.

Models for predicting emergence and abundance of Asian longhorned beetle. Native to China, the Asian longhorned beetle (ALB), *Anoplophora glabripennis*, is considered to be among the world's most devastating invasive species of hardwood trees, especially maples, and could potentially kill over 30 percent of all trees in areas of the eastern United States; a threat of approximately $670 billion. Early detection of and rapid response to ALB infestations requires that the implementation of surveys and control methods be precisely timed. Paradoxically, the adaptation of ALB to the varying climatic conditions from southern Canada to the United States is significantly compromising

researchers' ability to predict the optimal timing to implement surveys and control methods. In cooperation with the University of Delaware, ARS researchers in Newark, Delaware, developed degree day models for predicting adult ALB emergence from infested trees and estimates of how long these adult beetles survive. These models have resulted in earlier (by 2-3 weeks) survey and monitoring, are used in timing the deployment of attractants for early detection of adult beetles, and will be used to time the release of natural enemies for biological control of the beetle.

Control strategy mitigates the threat of the invasive Argentine cactus moth in North America. Subsequent to its detection in south Florida in 1989, the Argentine cactus moth had two incursions in Mexico, expanded its range along the Atlantic Coast and west along the Gulf Coast to the barrier islands of Mississippi and bayous of Louisiana, and became an imminent threat to many *Opuntia* cactus species, which are valued as a food, forage, wildlife habitat, and a major plant group in ecosystem structure and biodiversity. Following the successful eradication of this pest from Mexico, using control tactics including the sterile insect technique, ARS scientists in Tifton, Georgia, in collaboration with those in Tallahassee, Florida, and USDA, APHIS transferred mass-rearing technology to the Florida Department of Plant Industries Laboratory in Gainesville, Florida, and assisted in the establishment of an insectary supported by USDA, APHIS to supply sterile moths for the U.S.-Mexico bi-national cactus moth program. To insure moth quality, laboratory and field bioassays were developed and conducted on sterile moths and the data were used as feedback mechanisms to make protocol changes in both rearing and handling that improved moth quality and performance. USDA, APHIS and SAGARPA, Mexico continue to use these methods and tactics in their operational programs, which are part of an ongoing U.S.-Mexico bi-national campaign against this invasive pest to mitigate further westward expansion and population outbreaks.

PROBLEM STATEMENT 3B: TERRESTRIAL, AQUATIC, AND WETLAND WEEDS

Ecologically-based invasive plant management of invasive weeds in the western United States. In the United States, exotic invasive weeds not only cause an annual economic loss of about $35 billion, they also have environmental impacts ranging from the displacement of valued native species to the disruption of ecosystem functions. Cheat grass (CG) and medusahead rye (MHR) form an invasive species complex in the western United States that pushes out native vegetation, lowers the value of range for grazing, and creates a significant fire hazard. An areawide project conducted by ARS scientists in Burns, Oregon, has completed five years of effort to design ecologically-based invasive plant management (EIBM) against these species. These methods have been implemented throughout the western part of the United States and have served as a model for foreign countries with similar problems. The EIBM is a systems management approach that has classified the problem into separate, actionable problems. One of the considerations is repopulation of the landscape with native, desirable vegetation – a process that requires its own investment and techniques. The areawide project has also developed a "one pass" system that simultaneously removes the weeds with herbicides and seeds the area with native grasses. These methods have been communicated to those who can implement them. First, curricula were developed for professional students of rangeland

management, working with university partners to develop online course segments. Second, a Website was designed, keeping in mind the needs of different user groups; the site has an average of 400 users per month. Finally, printed guides, calendars, and other communication tools have been distributed and made available for download. This approach also holds promise for biological control agents of MHR, when applied to the planned exploration of southern Europe, the home range of MHR, by ARS scientists in Montpellier, France. This areawide project has increased pasturage and wildlife habitat in a large area of the United States and is a model for management of other invasive species that threaten environmental balance.

Component 4: Protection of Postharvest Commodities and Quarantine

PROBLEM STATEMENT 4A: INSECT PESTS OF FRESH COMMODITIES

Cold treatment stops coffee berry borer. Green coffee, which is shipped around the world for custom blending and roasting, carries the risk of spreading coffee berry borer. ARS scientists in Hilo, Hawaii, tested the freezing tolerance of over 15,000 coffee berry borer insects at three different temperatures and determined the temperature and time at which they could control 100 percent of all life stages. Hawaiian State regulators are using this information to implement a freezing treatment protocol that allows coffee growers in the infested area to ship green coffee to other islands without the need for methyl bromide fumigation.

Postharvest irradiation treatment controls light brown apple moth. Since the invasive, light brown apple moth (LBAM) was found in California in 2007, several countries have imposed trade restrictions on some of its host fruits and vegetables. ARS researchers in Hilo, Hawaii, found a radiation dose that resulted in zero tolerance (100 percent control) of the most tolerant LBAM stage. Some countries require zero tolerance to access their markets. This information will facilitate the trade of commodities that are hosts of LBAM.

PROBLEM STATEMENT 4B: INSECT PESTS OF DURABLE (STORED AND PROCESSED) COMMODITIES

Key discovery for effective microbial control of insect pests. One of the key elements of modern agriculture is effective control of insect pests. Without effective integrated pest management, overall crop production would likely decrease by 50 percent. Use of insect pathogens to control pests could have many advantages over other methods including a lower cost, elimination of the risk of environmental damage, and increased sustainability. Up until now, microbes have not been extensively used for pest control, except through extraction of the toxins some produce. ARS scientists in Manhattan, Kansas, worked with stored products pests to show that the normal gut bacteria of most insects act like a pro-biotic, protecting the insects from pathogenic microbes, and forms a barrier that hinders pathogen penetration of the gut wall. This discovery will lead to more effective microbial pesticides, a corresponding reduction in the amount of pesticides used, and lower crop production costs; as well as providing sustainable solutions for control of insect pests.