Veterinary, Medical, and Urban Entomology (National Program 104)
Annual Report for FY 2013

2, 4, 6, 8: Protecting two- and four-legged animals from the six- and eight-legged arthropods

Background:

National Program 104 (NP 104) includes projects that work toward better solutions for protection of humans and food animals from damaging arthropods. Although the program concentrates its efforts on priority pests, it covers a wide variety of topics, especially in veterinary entomology. Forty-six permanent scientists in 11 projects work on ticks, flies (mainly house flies, stable flies, and New World screwworm flies), mosquitoes, sand flies, biting midges, ants, and bed bugs. There are nine laboratories involved: Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland; Imported Fire Ant and Household Insect Research Unit, Gainesville, Florida; Mosquito and Fly Research Unit, Gainesville, Florida; Biological Control of Pests Research Unit, Stoneville, Mississippi; Agroecosystem Management Research Unit, Lincoln, Nebraska; Arthropod Borne Animal Diseases Research Unit, Manhattan, Kansas; Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville and Mission, Texas; Screwworm Research Unit, Pacora, Panama and Kerrville, Texas; and European Biological Control Unit-Greece, Thessaloniki, Greece. In addition, significant funding comes from NP104 for support of insecticide and application studies at the Natural Products Utilization Research Unit, Oxford, Mississippi, the Areawide Pest Management Research Unit, College Station, Texas, the IR-4 Project (minor use pesticide registration) at Rutgers University, and the Navy Entomology Center of Excellence in Jacksonville, Florida.

Scientists in NP 104 wrote 115 peer-reviewed scientific articles during this reporting period, 10 more than last year despite an additional 7.8% reduction in the program during the year due to mandated across-the-board funding decreases. The year also saw progress in translating research into practical products with 13 active Cooperative Research and Development Agreements (three new ones), 5 Material Transfer Agreements, 6 invention disclosures, and 6 patent applications. These products included better insecticides, better formulations, better traps, and better repellents. The program continued its cooperation with important stakeholders. Working closely with the U.S. Environmental Protection Agency, scientists provided expert advice on bed bugs, repellent labeling, and ticks. Our research provides the USDA Animal and Plant Health Inspection Service with direct research support of its imported fire ant biological control program, its Cattle Fever Tick Eradication Program, and its Screwworm Eradication Program. Discussions were also held with a wide variety of organizations in industry, agriculture, and academia.

The Deployed Warfighter Protection Program continued in its 10th year, providing $3 million per year in exchange for research directed at development of products for protection of military personnel from insect-transmitted diseases. The program is administered by the Armed Forces Pest Management Board, funded by the Department of Defense. Some of these funds are used to support insecticide and application studies at the Natural Products Utilization Research Unit, Oxford, Mississippi, the Areawide Pest Management Research Unit, College Station, Texas, the IR-4 Project (minor use pesticide registration) at Rutgers University, and the Navy Entomology Center of Excellence. Internationally, NP104 has continued work with partners in Albania, Argentina, Australia, Brazil, Canada, China, Costa Rica, Denmark, French Polynesia, Greece, India, Italy, Japan, Mexico, New Zealand, Panama, South Africa, Spain, Switzerland, Taiwan, Turkey, Ukraine, and the United Kingdom. Our aim is to form real partnerships that have benefit to the United States and to cooperating countries. These relationships not only give us access to places where many of our problems originated, it also increases the depth of our intellectual capital with original ideas from different perspectives.

NP 104 Events in 2013:

We welcomed the following new scientist to NP 104:

Randy Luttrell is the new research leader for the Biological Control of Pests Research Unit, Stoneville, Mississippi, expanding his leadership of the Southern Insect Pest Management Research Unit.

We wish the following NP104 scientists well in new endeavors:

Andrew Li, who transferred from Knipling-Bushling U. S. Livestock Insects Research Laboratory, Kerrville, Texas, to Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland, where he will be working on the prevention of Lyme disease through tick control.

We congratulate:

Center for Medical, Agricultural, and Veterinary Entomology, Mosquito and Fly Research Unit, Gainesville, Florida

Ken Linthicum for being recognized as a Finalist for the Samuel J. Heyman National Security and International Affairs Medal from the Partnership for Public Service and for being elected Vice President of the American Mosquito Control Association.

Dan Kline for being elected Vice President of the Society of Vector Ecologists.

Chris Geden for being elected MUVE (Medical, Urban, and Veterinary Entomology) representative to the Governing Board of the Entomological Society of America.

Knipling Bushland U.S. Livestock Insect Research Laboratory

Felix Guerrero for selection to be on the Fulbright Specialist Roster by the U.S. Department of State's Bureau of Education and Cultural Affairs and the Institute of International Education's Council for International Exchange of Scholars

John Goolsby and **Adalberto Pérez de León** for hosting a faculty member from the University of Texas-Pan American who was selected as a Fellow of the USDA-Hispanic Serving Institutions Kika de la Garza Program

Adalberto Pérez de León for appointment as Adjunct Professor in the Pathobiology Department of the Texas A&M University College of Veterinary Medicine & Biomedical Sciences in College Station, Texas; and to **Robert Miller** for appointment as Adjunct Professor at the University of Texas-Pan American in Edinburg, Texas

Weste Osbrink for appointment to a scientific advisory panel of the Environmental Protection Agency, for the improvement of performance standards required for registered pesticides

Mat Pound for being awarded a patent "Automated Injection System" which described a machine for injection of wild deer

Allan Showler for receiving the award for best presentation at the annual meeting of the American Society of Sugar Cane Technologists (ASSCT) in Panama City, Florida.

Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland

Andrew Li was sponsored by USDA Foreign Agriculture Service to travel to Russia to assess involvement of soft ticks (*Ornithodoros* spp.) in the outbreaks of African Swine fever

University of Florida, Emerging Pathogens Institute (collaborator)

Jeff Bloomquist had two students who won honors at the American Chemical Society's meeting in September 2013, in Indianapolis, Indiana. Daniel Swale (Ph.D. December 2012) won the Young Scientist Award, and Lacey Jenson (Ph.D. student) won best student poster. Daniel's research was on the DEET-octopamine receptor and Lacey's poster on cell-based high throughput screening studies.

Notable Accomplishments

DEET mosquito repellent works by taste as well as by smell

Current mosquito repellents are effective if used correctly, but dosages applied to the skin are very high. Prevention of disease transmission using repellents is a problem because people are reluctant to use unpleasant products until they have already been bitten.

Ideally, a repellent active ingredient would be developed that functions at the kinds of concentrations usually observed in the pharmaceutical industry – approximately 1000 times more effective than DEET. ARS scientists at Beltsville, Maryland, have been working to understand the physiological mode of action of repellents. Unexpectedly, they found that DEET not only affects odor receptors on the antennae, but also taste receptors on the mouthparts. The new understanding that taste and odor are involved in the function of this effective repellent opens the door to more precisely targeted exploration for repellent active ingredients that work at much lower concentrations. This discovery will change the way that new repellent active ingredients are evaluated and ultimately produce highly effective products that have a greater chance of preventing infection than current repellents.

Gene silencing technology leads toward safe mosquito control

There are a very limited number of public health pesticides available for controlling medically important vectors, such as mosquitoes and sand flies. This novel approach is based on the technology that allows for the specific silencing of genes critical to survival of the target vector species. This technology uses double stranded RNA (dsRNA) and the process of RNA interference (RNAi) to prevent the synthesis of specific proteins in cells. By choosing the right target, it is possible to debilitate mosquito vectors of disease. ARS researchers in Gainesville, Florida, have demonstrated that a dsRNA construct targeting a gut-expressed gene effectively shut down production of that protein when fed to the adult yellow fever mosquito. The dsRNA was fed to the mosquitoes in a sugar meal and stopped the protein in 12 or 24 hours. Oral delivery of dsRNA to mosquitoes could be a very practical way to deliver this new technology. The specificity of the dsRNA would prevent any danger to other insects, including pollinators. Also, only tiny quantities of dsRNA would be required, reducing costs. This work will lead to products that have no non-target effects and that are perfectly safe for humans and the environment.

How house flies live in bacteria-rich habitats

House flies breed in environments such as garbage and manure, where both maggots and adults are exposed to numerous species of bacteria. Many of those bacteria are human or animal pathogens. Remarkably, house flies are seldom harmed by the soup of bacteria in which they live. The interaction of the fly's own immune system and the bacteria in the environment and ingested by adult flies underlies the fly's ability to acquire, harbor and transmit bacteria to livestock and humans. In collaboration with Clemson University, ARS scientists in Manhattan, Kansas, sequenced genes that were activated in immune-stimulated house flies, and identified several sequences that coded for molecules that protect the flies from bacteria. Production of these substances allows house flies to live and thrive in contaminated environments. The sequences of these genes induced by immune challenge have been made public. An understanding of the intricacies of the relationship between flies and bacteria will lead toward better ways of protecting food and livestock from pathogens.

Viruses as powerful new way to control fire ants

Fire ants have become a problem in the United States and Asia because they do not have the complex of natural enemies that keeps them in check in their native South America.

ARS researchers at Gainesville, Florida, have worked with the Foundation for the Study of Invasive Species, Hurlingham, Argentina, for years to find and import small flies that parasitize fire ants. Those efforts are associated with an approximately 30% reduction of fire ants in Florida and Georgia. More recently, the two laboratories discovered the first viruses in any ant, one of which is particularly deadly for fire ants. This virus can be administered in bait and then sustains itself in a population through natural transmission. Adaptation of this product to area-wide programs will further reduce fire ant populations in the United States and lessen the impact of their range expansion associated with climate change.

Sequencing the genome of the cattle fever tick
Two species of cattle fever ticks that transmit babesiosis used to be responsible for the death and poor yield of cattle throughout the southern United States. They remain an important problem throughout the tropics. Beginning in 1912, a systematic program of dipping cattle and quarantining pastures totally eradicated the tick from the United States. The ticks are kept out of the United States through border inspection, inspection and treatment on farms in South Texas, and interception of infested cattle that wander across the border. The current acaricide used in the program is one of the few remaining organophosphates still on the market. Alternatives exist, but in Mexico there is resistance to any one of them. ARS scientists at Kerrville, Texas, in collaboration with researchers at Murdoch University, Perth, Australia, and the National Center for Genome Resources, have sequenced the genome of the cattle tick. Data from the genome sequence has resulted in the identification of anti-tick vaccine candidates that should provide an effective tool for the supplementation of acaricides. The sequence will also be valuable for rapid development of new acaricides targeted at new physiological processes. This is expected to accelerate acaricide research and development within USDA and the global animal health industry.

Biting midge gene expression
Biting midges are important vectors of livestock diseases, including some that have recently emerged. The northward extension of bluetongue virus in Europe and the appearance of Schmallenberg virus suggest that changing conditions may be increasing the importance of biting midges as vectors of animal diseases. In addition, biting midges transmit epizootic hemorrhagic disease, a deadly syndrome that threatens the deer farming industry in much of the United States. Because of the difficulty of working with these small insects, relatively little research has been done on them compared to more prominent biting insects like mosquitoes. ARS scientists in Manhattan, Kansas, in collaboration with Clemson University Genomics Institute, have worked to observe the genetic mechanisms of biting midges responsible for key biological processes. Looking at gene expression, they have discovered genes involved in blood feeding, defense, digestion, transport of materials within the insect, development, and reproduction. Pinpointing the role genes play in the life of the midge will contribute toward faster, more accurate assessment of vectorial competence and may lead to completely new modes of action for insecticides.

Chromosomal origin of fire ant social forms revealed
Introduced fire ants exhibit two colony social forms that differ in the number of reproductive queens per colony, as well as many other traits. Remarkably, many individual and life-history traits associated with social organization are correlated with a single gene, Gp-9. ARS researchers at Gainesville, Florida, found that Gp-9 is embedded in a group of genes that are resistant to reassortment and that therefore tend to stay attached to each other (i.e., a supergene). The pair of supergenes that make up the two alleles of an individual are located on separate, dissimilar chromosomes. Most genes associated with social forms of the ants are located on these two chromosomes, which are similar to sex chromosomes in structure. These findings highlight how genomic rearrangements can maintain divergent adaptive social phenotypes involving many genes acting in concert by locally limiting recombination. This understanding of basic gene mechanics has great scientific interest and may also lead to better ways to alter the social forms of these invasive ants.

A viral biopesticide against house flies
ARS researchers at Gainesville, Florida, have attempted for years to infect flies with salivary gland hypertrophy virus (SGHV) with baits. Even though flies fed on virus-laden food, they failed to be infected. The scientists unexpectedly found that surface contamination may be a more common route of infection, and that even small amounts of cuticular damage to the fly can provide routes of infection without ingesting the virus. A mathematical model of virus epizootiology was developed that suggested that the aggressiveness of male flies during courtship could provide an additional avenue of infection. These findings support the idea that a residual spray of the virus could find its way into house flies, providing a new and safe way to control these insects.

Development of a new, selective insecticide
One of the objections to pesticides is their toxicity to organisms, including humans, not intended as their targets. Although residue limits based on toxicological data protect people, compliance with those limits can be a problem. From an environmental perspective, toxic effects on any vertebrate species would be considered undesirable. ARS scientists at Kerrville, Texas, collaborated with researchers at the University of Florida and Virginia Tech to evaluate a synthetic carbamate insecticide, designated PRC-408, for the control of horn, stable, and sand flies. PRC-408 was as toxic to these insects as carbaryl, an efficacious, commercially available compound. An *in vitro* assay was used to demonstrate that PRC-408 exhibited approximately 300-fold higher specificity for its arthropod target compared to its mammalian, i.e., bovine and human, target and offers improved safety compared to other chemicals in its class. This work will result in new insecticides that are very safe to use, but flexible in their application.

Desert treatments of biting insects
ARS researchers at Gainesville, Florida, and their collaborators have recognized the insect control problem faced by the U.S. military and others who live in hot, dry deserts. The hot desert floor tends to disperse insecticidal fogs through convection, dispersing any insecticidal fog applied. Trials in California's Coachella Valley showed that mosquitoes in cages were killed despite the unfavorable conditions. They also showed that droplets

of the insecticide, permethrin, deposited adequately on surfaces. The results indicate that despite unfavorable conditions for traditional aerial ULV application, aerial application of insecticidal fogs against medically important mosquitoes can substantially reduce mosquito-human contact and minimize transmission of mosquito-borne viruses to military troops deployed in desert areas.

Rift Valley fever is more complicated than we thought

Rift Valley fever is caused by a virus that can be transmitted by mosquitoes or by direct contact. It can be a serious disease for people and animals, occurring in outbreaks at irregular intervals in Africa. About ten years ago it appeared outside of Africa for the first time, which raised the alarm that the virus might be able to cause disease elsewhere. ARS scientists in Gainesville, Florida, and Manhattan, Kansas, worked with the Kenyan researchers to examine blood sera of many animals sampled during a Rift Valley fever outbreak. They found that prevalence of positive antibodies in domestic and wild animals occurred at the places and times predicted by a model developed jointly by ARS and NASA. Studies in Africa showed unexpected transmission by *Mansonia* mosquitoes. In the laboratory, *Psorophora* and *Coquillettidia* were also shown to be competent vectors of the virus. Since some of these species live in the United States, it appears that Rift Valley fever could become endemic in the United States, as happened with West Nile virus. The consequences of the establishment of Rift Valley fever in the United States would be far more serious than for West Nile, because of Rift Valley fever's devastating effects on livestock and as a cause of serious human disease. This research gives veterinary and human health a more accurate risk assessment of Rift Valley fever.

The difference between red and black fire ants

The red imported fire ant, *Solenopsis invicta*, is one of the most successful invasive ants in the world. Native to South America, *S. invicta* has been introduced into many countries and regions. The black imported fire ant, *Solenopsis richteri*, a closely related species to *S. invicta*, is also an invasive ant but with much less success. What makes the red imported fire ant so successful? The fire ant scenario in the United States provides a unique opportunity to examine this question. Both red and black imported fire ants invaded the United States through the same entrance: the port of Mobile, Alabama. *S. richteri* arrived in about 1918 and *S. invicta* in 1930s. Although *S. richteri* was introduced and established more than one decade earlier than *S. invicta*, the latter has gradually displaced *S. richteri* throughout most of its distribution area. Both *S. invicta* and *S. richteri* belong to the *saevissima* species complex. They share many traits. However, some less noticeable differences between *S. invicta* and *S. richteri* influence their different invasion success. ARS scientists at Stoneville, Mississippi, evaluated stress tolerance for both ant species. *S. invicta* was more tolerant to toxicants than *S. richteri*, due to significantly higher level of detoxifying enzyme activity in *S. invicta*. *S. invicta* was also significantly less vulnerable than *S. richteri* to both heat and desiccation stress. Despite *S. richteri* having significantly higher body water content, *S. invicta* was less sensitive to desiccation stress due to its significantly lower water loss rate. High tolerance to abiotic stresses may contribute to the success of red imported fire ants.

National Program 104 Annual Report 2013: Appendix

Leveraging Resources

Center for Medical, Agricultural, and Veterinary Entomology, Imported Fire Ant and Household Insects Research Unit (IFAHIRU), Gainesville, Florida

DeWayne Shoemaker: funding from the National Science Foundation to identify targeting sequence data within several species of fire ants in cooperation with the University of Georgia

Sanford Porter: funding from the Galapagos Conservancy to allow for release of decapitating phorid flies against tropical fire ants in Hawaii, Guam, and other U.S. protectorate islands and for receiving continued support from USDA Animal and Plant Health Inspection Service (APHIS) to facilitate rearing, evaluation, and field release of new species of fire ant decapitating phorid flies

David Oi: continued support from USDA APHIS for development of fire ant quarantine treatments to protect nursery stock and for receiving funding from Coachella Valley Mosquito and Vector Control District in order to establish biological control agents against imported fire ants

Robert Vander Meer: established a Material Transfer Research Agreement with the National Taiwan University, Taiwan, that will facilitate release of fire ant biological control agents developed at IFAHIRU in Taiwan

Steven Valles and **Robert Vander Meer:** continued support from USDA APHIS in order to develop an automated surveillance system and in-field test for identifying imported fire ants

Knipling-Bushland U.S. Livestock Insects Research Laboratory

John Goolsby: $3 million for extension of Trust Agreement with the Department of Homeland Security for development of biocontrol of *Arundo donax* and for leading a group of scientists, including collaborators from India, that conducted the first exploration program for biological control agents of *Rhipicephalus microplus* in its native range in India and the Philippines

Pia Olafson and **Kim Lohmeyer:** funding from the National Cattlemen's Beef Association to evaluate the role of larval and adult biting flies as reservoirs/vectors for *Salmonella*