Veterinary, Medical, and Urban Entomology (NP 104)

Annual Report for 2012

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

Background:

National Program 104 (NP104) 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. Fifty-five permanent scientists from 9 laboratories in 13 projects work on ticks, flies (mainly house flies, stable flies, and New World screwworm flies), mosquitoes, sand flies, biting midges, ants, and bed bugs. In addition, significant funding comes from NP104 for support of insecticide and application studies at two ARS laboratories, the IR-4 Project (minor use pesticide registration) at Rutgers University, and the Navy Entomology Center of Excellence.

Scientists in NP104 wrote 105 peer-reviewed scientific articles during this reporting period, six more than last year despite a 25% reduction in the program during the last two years. The year also saw progress in translating research into practical products with 15 active Cooperative Research and Development Agreements, five Material Transfer Agreements, eight invention disclosures, three patent applications, and one product licensed. 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 testing, 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 ninth 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 of insecticide and application studies at the Natural Products Utilization Research Unit, Oxford, MS, 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.

This reporting period saw the end of the Formosan Subterranean Termite Research Unit, which was closed as part of cost cuts in the Agency. The unit was closed because it had solved the problem for New Orleans, providing the means to save the historical wooden

buildings of the French Quarter from destruction. As a result of this closure and others, a number of entomologists were transferred into the remaining laboratories.

Internationally, NP104 has continued work with partners in Australia, Brazil, Argentina, Mexico, Panama, Costa Rica, Ecuador, Kenya, China, Switzerland, New Zealand, Egypt, France, Greece, Albania, and Turkey. 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 2012:

We welcome the following new scientists to NP 104:

Robert Pfannenstiel (transferred from Weslaco) and Mark Ruder at the Arthropod Borne Animal Diseases Research Unit (ABADRU), Manhattan, Kansas.

Kristina Friesen located at the Agroecosystem Management Research Unit, Lincoln, Nebraska.

Allan Showler (transferred from Weslaco) and Weste Osbrink (transferred from New Orleans) to the Knipling-Bushland U.S. Livestock Insect Research Laboratory (KBUSLIRL), Kerrville, Texas.

John Goolsby and Donald Thomas (both transferred from Weslaco) to the Cattle Fever Tick Laboratory (a satellite laboratory of the KBUSLIRL) in Mission, Texas.

Margaret Allen located at the Biological Control of Pests Research Unit, Stoneville, Mississippi.

Mary Cornelius (transferred from New Orleans) located at the Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

We wish the following NP104 scientists well in new endeavors:

Alan Lax, who retired from the Formosan Subterranean Termite Research Unit (FSTRU), New Orleans, Louisiana.

Matthew Tarver, who transferred from the FSTRU to the Honey Bee Breeding, Genetics, and Physiology Research Unit, Baton Rouge, Louisiana.

Dunhua Zhang, who transferred from the FSTRU to the Aquatic Animal Health Research Unit, Auburn, Alabama.

Maureen Wright, who transferred from the FSTRU to the Commodity Utilization Research Unit, New Orleans, Louisiana.

Kumudini Meepagala, who transferred from the FSTRU to the Natural Products Utilization Research Unit, Oxford, Mississippi.

John Bland, who transferred from FSTRU to the Food Processing and Sensory Quality Research Unit, New Orleans, Louisiana.

Beverly Wiltz, who left FSTRU and ARS.

We congratulate:

Diane Kammlah (KBUSLIRL) for receiving a USDA APHIS Award of Appreciation for her contributions to the Cattle Fever Tick Eradication Program.

Robert Miller (KBUSLIRL) for deployments as a Naval Reserve officer.

Mark Feldlaufer (IIBBL) and Daniel Strickman (ONP) for a U.S. Environmental Protection Agency Bronze Medal in recognition of service on the Bed Bug Federal Task Force.

Muhammad Chaudhury (SRU) for recognition by Mississippi State University for his years of contributions to their Insect Mass Rearing Workshop.

Juan Morales-Ramos of the Biological Control of Pests Research Unit for a Federal Laboratory Consortium Award for Excellence in Technology Transfer entitled "In Vivo Production of Entomopathogenic Nematodes."

Xixuan Jin of the Biological Control of Pests Research Unit for a Federal Laboratory Consortium Award for Excellence in Technology Transfer entitled "Method for Encapsulation of Microparticles."

Notable Accomplishments:

Transgenic screwworm produces only males

The screwworm fly is a damaging pest of livestock that infests wounds and eats living flesh, often killing cattle and other animals. The screwworm fly used to live throughout the southern United States, but was eradicated by the systematic release of sterile male flies that mated with wild female flies, a procedure developed by ARS. Between 1953 and 2005, the program administered by APHIS successfully eradicated the fly from all of North and Central America. Currently, the fly is prevented from re-infesting these areas by continuously releasing sterile males in eastern Panama as a barrier between South America, where the fly still exists, and Central America, where it has been eradicated. Screwworms are produced in a large factory in Panama, supported by the ARS research program. For the past five years, APHIS funded ARS to develop a transgenic strain of screwworm fly that would only produce males, saving rearing, distribution, and sterilization costs. This project required discovery and insertion of a DNA cassette that

was female sex-linked and lethal when tetracycline was not added to the larval medium. In August, 2012 ARS scientists in Kerrville, Texas, and Pacora, Panama, worked with a collaborator at North Carolina State University to successfully produce a strain in which 99% of females died if tetracycline was not present in the medium. The patient search for the right DNA sequences and the methods to both transform and rear the screwworms produced the strain only a few months later than projected. This strain will allow ARS to prove the concept of the value of a transgenic, male-only strain, eventually saving APHIS as much as $5 million per year.

Practical control of sand flies for the military

Old world sand flies are responsible for the transmission of leishmania to humans in areas where U.S. military personnel are currently deployed. Researchers at ARS in Gainesville, Florida, successfully conducted the first study examining the efficacy of Ultra-low volume (ULV) pesticide applications on Old World sand fly species in leishmaniasis-endemic regions in a natural setting in Africa. These findings indicate that by combining ULV treatments of natural sand fly populations with treatment of camouflage netting, permethrin-treated clothing, and the use of DEET could substantially minimize human-sand fly contact and decrease transmission of leishmania to deployed troops. The outcome of this research leads to disease risk reduction for U.S. military personnel.

Accurate mosquito trapping results for precise integrated pest management

Integrated pest management requires accurate information on the number of pests and where they are. This is particularly important for mosquito control because resources are always limited compared to the geographic area requiring treatment. Scientists at ARS in Gainesville, Florida, compared the number of female mosquitoes captured by suction traps, portable light traps (with carbon dioxide), and landing on a human subject. The results of this research showed that mosquito density from light trap samples was underestimated by 43-97% and over-estimated by 80-85% (depending on species), when compared with the mosquito landing rate on a human subject. Corresponding values for suction trap samples were 28-88% and 17-87%. A statistical algorithm that corrects mechanical trap-based estimates of adult mosquito density to the landing rate of mosquitoes on a human host was constructed. Use of this algorithm will provide a better estimate of mosquito density in a local area which in turn can be used to help mosquito control professionals determine the best method of mosquito control. The algorithm will also enable state and federal authorities to more accurately compare trapping results from different locations. More accurate estimates of mosquito numbers will help authorities target resources, make accurate risk assessments of disease, and assess confidence whether or not invasive species are present.

Novel and effective vaccine for cattle against the cattle fever tick

The southern half of the United States used to be infested with two species of one-host tick that transmitted bovine babesiosis to cattle. The disease is often fatal in adult cattle and is one of the infections that prevent export of live animals. Between 1912 and 1943, the USDA eradicated both species of ticks by systematic programs to dip all infested cattle in pesticide. The ticks are abundant in Mexico and elsewhere in the word, so that

APHIS actively fights re-introduction by requiring special treatment of cattle from areas where the ticks still live and by maintaining a quarantine zone in southern Texas between Mexico and the United States. Anti-tick vaccines are an attractive idea for control, both to reduce the amount of pesticide required in eradication programs and to manage tick populations overseas where the ticks continue to be a problem. A vaccine based on a tick gut protein, Bm86, has been available in some countries for over ten years. Recent evaluations performed by ARS in Mission, Texas, in cooperation with APHIS showed that the Bm86 vaccine is highly effective against one of the species of cattle fever ticks and ineffective against the other. Genomic studies and bioinformatics of the cattle fever tick by ARS scientists in Kerrville, Texas, produced a series of vaccine candidates based on finding protein sequences likely to cause a strong immune response in cattle. ARS scientists at Kerrville, Texas, in collaboration with EMBRAPA scientists in Campo Grande, Brazil, completed trials of the most promising candidate vaccine antigens. One potential vaccine was 75% effective against the cattle fever tick that was unaffected by Bm86. The mechanism of action of the vaccine was demonstrated by knocking out the target tick gene with RNAi, showing that that particular gene was essential for tick survival. This novel antigen was highly expressed in tick nerve tissues, and the results indicate that vaccination against cattle ticks has great potential for integration into APHIS' Cattle Fever Tick Eradication Program and for tick management in contribution toward international food security.

Treatment of stable fly larval sites using an organic compliant chemical

Stable flies develop in damp soil with an abundance of incorporated vegetable matter, conditions often associated with feed lots and other cattle operations. The flies feed on the blood of many kinds of animals, including humans. They are considered the principle pest of cattle in the United States, causing $2 billion per year in loss of yield according to a recent estimate. Larval control has been difficult because of the widespread distribution of maggots and the difficulty of applying chemicals to them below ground. An encapsulated formulation of catnip oil was developed by ARS scientists in Lincoln, Nebraska, to control immature stable flies developing in animal wastes. The formulation deters female flies from depositing their eggs and inhibits larval development under field conditions. A single application is effective for 5-7 days. This is the first botanical-based product for the control of immature stable flies. The formulation, once registered, will provide an effective stable fly control option for organic cattle producers.

Bed bugs shown to affect human health

Bed bugs are a blood-sucking pest that lives in homes, hotels, shelters, vehicles, and businesses. The bugs feed at night causing a variety of reactions from mild irritation to extensive blistering and allergic reaction. Bed bugs had been controlled effectively in the United States since the 1950s until they became much more numerous starting in approximately 2002. They are now a chronic problem in many American cities, especially affecting hotels, shelters, and multi-family homes. In response to stakeholder input in 2007, ARS started a program to study chemical ecology and control of bed bugs at Beltsville, Maryland. ARS participation in the Federal Bed Bug Task Force has facilitated extensive discussions with other agencies, including the CDC. Although the CDC participates, it does not consider bed bugs a public health pest because they do not

transmit any pathogens. ARS scientists worked in collaboration with The University of Mississippi Medical Center and Harvard Medical School to show that bed bug bites can cause a very severe, localized inflammation of blood vessels. Moreover, the type of inflammation that occurs has the potential to affect major body organs. These data show that bed bugs are not only an annoyance, but also a health threat. The impact of this research may be greater involvement by proponents of public health in control of the national bed bug problem.

Supergene Gp-9 associated with multiple mating and male reproductive successes in fire ants

Understanding fire ant male reproductive success and fitness are important components of research aimed at suppressing fire ant populations, yet data on this important topic are virtually nonexistent. ARS researchers in Gainesville, Florida, conducted a study aimed at determining how commonly fire ant queens mate with more than one male. This study revealed that some fire ant queens mate with more than one male and that whether a queen mates with more than a single male is determined almost entirely by male genotype. Investigation of the physiological basis for the inability of some males to discourage a second mating revealed that male sperm count also is linked to male genotype, suggesting fire ant queens remain receptive to mating if their first partner does not provide a sufficient quantity of sperm. Understanding the importance of the male genotype in fire ant colony structure is an important basic discovery that uncovers a new pathway for disruption of this invasive species.

Progress in biological control of fire ants

Imported fire ants are unusually abundant in the United States, probably because they have escaped their natural enemies left behind in South America. ARS researchers in Gainesville, Florida, have confirmed the establishment of the new phorid decapitating fly, *Pseudacteon cultellatus*, near Miami and in Gainesville, where it is beginning to expand out of the release area. This new species of fly specializes on attacking the smallest sizes of fire ant workers, which are most abundant in multiple-queen fire ant colonies. This preference is especially important because multiple-queen fire ant populations average 2-3 times the densities of regular single-queen fire ant populations and are therefore a substantially greater pest of homes, agriculture, and the environment. Another species of phorid decapitating fly, *Pseudacteon obtusus*, preferentially attacks larger fire ant workers. This phorid fly was shown to multiply well even in the presence of other species of decapitating flies. This is significant because elimination of the larger worker ants will have a greater negative effect on the colony.

Genomics and biochemistry of termites

The threat of the Formosan subterranean termite to the southeastern United States resulted in a robust research program at ARS on this pest in 1997. Through basic and applied research, cooperation with academic institutions, and a demonstration project, the program successfully solved the problem through areawide integrated pest management and literally saved the French Quarter of New Orleans from destruction. Scientific accomplishments continued through the last year of ARS' termite research, with particularly significant contributions on genomics and biochemistry. ARS scientists in

New Orleans, Louisiana, performed a project focused on sequencing the Formosan subterranean termite genome and its comparison to the native subterranean termite genome. This work was performed in collaboration with researchers at the J. Craig Venter Institute and Purdue University. The project completed 18x coverage of the 926 mb genome using Illumina Nextgen sequencing. In addition, ARS scientists identified and characterized a novel endogenous endo-ß-1,4-glucanase (named CfEG5) in the Formosan subterranean termite. Eleven of 15 genes belonging to the metabolic mevalonate pathway were identified. This pathway is responsible for the production of the morphogenic hormone, juvenile hormone, which controls caste differentiation. Further progress was made in understanding caste differentiation by identifying differences in the important musculo-neural protein, myosin, between worker and soldier termites. Basic studies of the biochemistry of carbohydrate metabolism led to discovery of an inhibitor that has promise as a safe pesticide. These basic and applied scientific accomplishments form a foundation for the next work on termites performed outside ARS.

Operational research in support of APHIS' Cattle Fever Tick Eradication Program
The Cattle Fever Tick Eradication Program depends heavily on dipping cattle in solutions of the organophosphate pesticide, coumaphos. An alternative developed in cooperation between ARS and APHIS involves injection of long-acting doramectin. Although injections of doramectin to eradicate cattle fever ticks require half the number of treatments as standard coumaphos dips and significantly reduces costs of regulatory treatments to ranchers, there is concern that repeated injections at 25- to 28-day intervals could eventually reduce efficacy of treatments. In a study at Edinburg, Texas, cattle were repeatedly injected at 28-day intervals throughout the year, with blood serum concentration used as a predictor of the probability of female cattle fever ticks being able to survive and reproduce by successfully feeding to repletion between treatments. Of the two dosages that were tested, the higher dose had a 100% kill rate, and the blood serum concentration never dropped below this level between treatments. Thus, at this dosage it would be impossible for ticks to reach full engorgement between consecutive treatments. Results of this study demonstrated that the trial policy, instituted by the USDA, APHIS, VS, Cattle Fever Tick Eradication Program, of repeatedly treating cattle with doramectin injections at 25- to 28-day intervals for eliminating cattle fever ticks would produce little or no risk of any viable ticks developing to repletion and re-infesting the field between treatment applications.

Mosquito variation across the nation
The United States is host to over 150 species of mosquitoes and each region has its own group of problem species. A few species occur across the country and are very important as vectors and pests. Two of those species are *Culex tarsalis* and *Aedes vexans.* The former species is a principle vector of West Nile virus in the western United States and the latter is a severe pest and occasional virus vector across the entire country. In spite of the importance of these species, their genetic population structure has never been thoroughly examined. ARS scientists at Manhattan, Kansas, have initiated a project to collect many different populations of these species and use the most recent genetic methods to determine population genetic structure. Those methods examine the entire

genome of individual specimens to make a thorough comparison of the degree of differences across the continent. More than 86 entities (individuals or agencies) collected 454 unique populations making this one of the largest coordinated collections of disease vector mosquitoes in North America. These mosquitoes will be used to determine the differences between populations of the two species with implications for how to control them and which populations are most likely to transmit pathogens.

Control of mosquito larvae with polyoxyethylene tridecyl ether

Surfactants have been used in soft bodied arthropod control for years, but little is known about the mechanism. ARS scientists at Stoneville, Mississippi, found that the combination of both the surfactant chemistry and its specific hydrophilic–lipophilic balance (HLB) number can create the best insecticidal activity against mosquito larvae and pupae. A chemical was found that is highly lethal to mosquito larvae and pupae. Oils are the only products available that kill pupae, but oils are difficult to use and sometimes damaging to plants. A soluable, pupicidal surfactant could prevent emergence of pupae and kill larvae in one step. This product may also be a valuable adjuvant for other pesticides, improving penetration of the cuticle.

A critical enzyme identified in a sand fly species

Sand flies transmit pathogens that cause a variety of diseases in humans, including verruga, kala azar, cutaneous leishmaniasis, and sand fly fever. Cutaneous leishmaniasis was a major problem for the U.S. military in Iraq and kala azar accounts for hundreds of thousands of childhood deaths in Africa. ARS scientists at Kerrville, Texas, identified, cloned, and sequenced sand fly acetylcholinesterase, an enzyme which is the target for many kinds of effective pesticides. Collaborative research with the University of Florida is identifying compounds that closely target sand fly acetylcholinesterase. Such active ingredients will not only be effective, but safe for people and other non-target organisms. These findings offer the opportunity to develop new insecticides for effective sand fly control.