Veterinary, Medical, and Urban Entomology (NP 104)

Annual Report for 2010

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

Background:

National Program 104 involves efforts in 11 units at 12 different locations performed by over 80 scientists. The subjects of investigation all concern improvements in integrated pest management, the pests including termites, ants, mosquitoes, biting midges, sand flies, biting and filth flies, screwworm flies, bed bugs, and ticks. We aim for 30% of our work to be basic, leading the way to true innovation for the management of pests and vectors. The other 70% of the work is focused on solution of particular problems, including developmental work beyond the research phase. In standard government parlance, we perform research at Phases I through III.

This year, NP 104 completed the first year of its new project plans. Most of these were a smooth transition from previous work, but others reflected some real changes in direction. Our program remains flexible and receptive to applying its efforts to new problems, especially at the specific request of stakeholders. For example, the USDA Animal Plant Health and Inspection Service (APHIS) made specific requests for changes in our research direction on screwworm flies, which has resulted in a shift in direction by some of our scientists. Those changes were based on real-world events in this operational program that keeps the United States and the rest of North America free of this dramatically damaging pest.

We have not seen any growth in our base funds during the year, but we have been able to find some additional soft funds for the program. Balancing the continuous need for additional funding with the maintenance of our core mission is a constant challenge and the resulting dialog is a big part of the innovative process. These funds have come from grants, other government agencies, and industry.

This year we saw the beginning of a new NP 104 program based at the USDA ARS European Biological Control Laboratory's satellite location in Thessaloniki, Greece. Funded by the Department of Defense' Deployed Warfighter Protection Program (DWFP), we will have a University of Florida post-doc stationed there to work on integrated management of sand flies. Sand flies have been the most important vector during nine years of combat operations in Afghanistan and seven years of operations in Iraq, but they have been difficult to control, especially in a hot desert environment. The post-doc will establish a working field site in Greece, with extension of more limited trials in the Middle East. We are hoping that this arrangement will be a cost-effective means of proving concept of a number of techniques for sand fly control developed by USDA ARS and DWFP.

As reported last year, NP 104 started bed bug research in response to stakeholder input. As the problem gained close public attention in 2010, we found ourselves to be the only federal agency with any active research on the subject. This work has only been in progress about 18 months, but it has already resulted in two peer-reviewed publications and the discovery of chemicals that affect these pests' behavior. The program at the Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland, is also screening newly discovered insecticides (most of which were developed by USDA ARS under the DWFP program). Although this program is funded by USDA ARS for only one investigator and one technician, it has pulled us into the center of the U.S. Environmental Protection Agency-led Federal Bed Bug Working Group and presentation to a Congressional forum.

Internationally, NP 104 has continued work with partners in Australia, Taiwan, Brazil, Argentina, Mexico, Panama, Kenya, and France. 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.

Many of the veterinary, medical, and structural pest problems in the United States continue in their usual status and our work makes progress in controlling them more efficiently. However, being part of biological systems, the pests and diseases are not static, but are constantly adjusting to conditions created naturally and by human activity. We see this especially with cattle fever ticks, which are now able to establish themselves independently from cattle by growing on white-tailed deer and probably other ungulates. We are also very concerned about the public health threat of the Asian tiger mosquito. This species continues to expand its range and abundance in the eastern United States and was responsible for both dengue and chikungunya virus transmission in Europe during 2010. Our best efforts to control this mosquito result in a reduction of only about 70% of the population, which may not be enough to prevent outbreaks of those diseases. National Program 104 works in partnership with Rutgers University to apply existing control methods more efficiently and to develop new ones.

NP 104 Events in 2009:

We welcome the following new scientists to NP 104:

Alexandra Chaskopoulo, Ph.D., as a post-doc at the European Biological Control Laboratory-Greece, Thessaloniki, Greece.

Lee Cohnstaedt, Ph.D., as a new scientist at the Arthropod Borne Animal Diseases Research Unit, Manhattan, Kansas.

Danett Brake, Ph.D., as a post-doc at the Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Samuel Liu, Ph.D., as a post-doc at the Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Liza Soliz, Ph.D. candidate, as wildlife biologist at the Cattle Fever Tick Research Laboratory in Mission, Texas through an agreement with APHIS-WS.

We congratulate:

Kamal Chauhan, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland, for receiving the 2010 Beltsville Area Technology Transfer Award and the 2010 Federal Laboratory Consortium Award for Developing Superlure for the biocontrol of the arthropod insect pests.

Ken Linthicum, Gary Clark, and Uli Bernier of the Mosquito and Fly Research Unit (MFRU), Gainesville, Florida, for being invited to speak at the 50th Anniversary of the World Health Organization Pesticide Evaluation Scheme (WHOPES) and receiving an award recognizing CMAVE's contributions to this program.

Ken Linthicum and Seth Britch from MFRU and colleagues from DoD, NASA, CDC, WHO, and FAO were awarded the USDA-ARS 2009 ARS Superior Effort Technology Transfer Award - *For outstanding effort and creativity in the development and transfer of Rift Valley fever outbreak early-warning system to protect global agriculture and public health.*

Ken Linthicum and Seth Britch from MFRU and colleagues from DoD, CDC, and NASA received the 2010 Federal Laboratory Consortium National Technology Transfer Interagency Partnership Award, Albuquerque, New Mexico.

Ken Linthicum from MFRU received the 2009 Department of Energy, Federal Energy Management USDA Energy Champion Pioneer Award.

Ken Linthicum from MFRU received the 2010 Federal Laboratory Consortium Laboratory Director of the Year Award, Albuquerque, New Mexico.

Daniel Strickman, Office of National Programs received the John Belkin Memorial Award from the American Mosquito Control Association for lifetime achievement in the study of mosquito biology and systematics.

John B. Welch, Ph.D., completed 25 years of combined service with USDA-ARS, including 3 years at the Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas. Dr. Welch accepted the position of Technical Director with the USDA-APHIS International Safeguarding Screwworm Eradication Program in Panama.

Joe M. Pound, completed 25 years of service with Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Felix Guerrero, completed 20 years of service with Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Technology Transfer:

One of the ways in which ARS scientists provide products that solve agricultural problems is through direct interactions with the public and industry. NP 104 managed four websites that provided information directly to those who need it. Inventions from the program resulted in one new patent applications, five invention disclosures, and eight active research agreements with industry, and nine material transfer agreements. The inventions that are most ready for development are advertised to industry as potentially valuable licenses (http://www.ars.usda.gov/business/availtechs.htm).

Specific non-proprietary technology transfer included:

- ‘2-Poster’ Deer Treatment Feeder Adapters.
- A new synergized formulation of insecticides for horn fly control.
- Improved technique for controlling the cattle tick and reducing the incidence of bovine babesiosis.
- Pre-fabricated leg bands for Scottish Red Grouse.
- Vaccination of animals to elicit a protective immune response against tick infestations and tick-borne pathogen transmission.
- Bibliography on the bed bug that has been made available to the public through the Armed Forces Pest Management Board (AFPMB) website.

Notable Accomplishments:

A million dollar savings by strategic releases of screwworm flies
The screwworm fly lays eggs in wounds and then burrows into the flesh of humans, cattle, and other mammals, often with fatal results. This horrific pest was eradicated from the entire continent of North America by systematic release of sterile males and a barrier of sterile males continuously released in Panama prevents reintroduction of screwworm fly from South America. ARS researchers of the Screwworm Research Unit in Kerrville, Texas, and Panama reviewed and analyzed release technologies to improve screwworm fly dispersal relative to where, when, and how many sterile flies are released in the barrier zone. Quantitative calculations were based on screwworm biology and modeling of results, as well as application of global information systems. Recommendations with supporting data presented to the Panama – U.S. Commission for

Eradication of Screwworm (COPEG) will (1) result in updated equipment on-board dispersal aircraft, (2) strategically reduce the number of flies dispersed, and (3) save up to $1,000,000 annually. Recommended improvements to the navigational software and equipment are currently being implemented by USDA APHIS International Services and COPEG, the agencies that operate the sterile male barrier zone.

Vaccines to stop the cattle fever tick

Cattle fever ticks are a world-wide pest of cattle that decimate the economics of the industry by exsanguination of animals and by transmission of key diseases like babesiosis and anaplasmosis. These diseases not only reduce the efficiency of production, they also prevent trade in live animals. The cattle fever tick is a current threat to international food security and an imminent threat to the cattle industry in the southern United States, where the tick was eradicated during a period between 1907 and 1943. ARS scientists in Kerrville, Texas, collaborating with scientists from EMBRAPA Brazil, identified two anti-cattle tick vaccine candidates in cattle trials. These candidates had been prioritized in a prior ARS project through bioinformatic and molecular biological approaches. In the cattle trials conducted in Brazil, the candidates outperformed the recombinant Bm86 Campo Grande antigen, which is an antigen similar to that used in the only current commercially available anti-tick vaccine. An invention disclosure was filed and cattle trials are scheduled to evaluate various parameters in the vaccination protocol to optimize efficacy. The worldwide use of a consistently effective anti-tick vaccine in cattle would reduce production costs associated with tick treatment and contribute to the maintenance of the eradication of cattle fever tick in the United States.

New methods for protecting U.S. troops deployed overseas

Protection of deployed military from mosquitoes and flies that transmit disease is a critical component of successful military operations because U.S. military personnel are generally susceptible to the pathogens and they are more exposed to bites in the field. ARS researchers from Gainesville, Florida, carried out field trials of two insecticides (bifenthrin and lambda-cyhalothrin) as barrier treatments (applied to vegetation or to military materials between the source of biting arthropods and the location to be protected) in desert environments (Coachella Valley, California; western Kenya, Iraq, and Afghanistan) and in a humid sub-tropical environment in Florida. Results from vegetation treatments indicated significant reduction in mosquitoes in field counts and lab assays for up to a month; results from material treatments indicated significant reduction of mosquitoes or sand flies in field counts and lab assays for up to 18 months. Another way to reduce the number of vectors is application of aerosols of insecticide outdoors. Trials in a desert environment showed that thermal fog (machines that burn a mixture of insecticide and oil to produce a dense insecticide-laden smoke) was superior to ULV (ultra-low volume, which produces very small droplets in a mist that is made mechanically) and that malathion, an organophosphate, was better than sumithrin, a pyrethroid. The results suggest that, as an enhancement to the current DoD pest management system (such as use of DEET and permethrin treatment of uniforms) barrier treatments may be successful in providing protection from vector-borne diseases for deployed troops in desert habitats by significantly reducing densities of mosquitoes or sand flies reaching individual personnel in protected areas.

Natural biological control of the imported fire ant
Fire ants, accidentally imported from Argentina in the early 20th century, are unusually abundant in the United States, because they have escaped their natural enemies left behind in South America. ARS researchers in Gainesville, Florida, obtained approval from the North American Plant Protection Organization (NAPPO) and USDA-APHIS regulators to release a new species of phorid decapitating fly (*Pseudacteon cultellatus*) as a fire ant biocontrol agent. 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 two to three times the densities of regular single-queen fire ant populations and are therefore a substantially greater pest of homes, agriculture, and the environment. Another example of significant progress in development of biological control was the comparison of a new fire-ant virus, SINV-3, from American and Argentinean populations. Working with the USDA ARS South American Biological Control Laboratory, ARS scientists sequenced the SINV-3 genome in entirety from an Argentinean source and compared it with the genome sequence found in U.S. populations. The Argentinean variant had a different genomic architecture and may exhibit different virulence levels compared with the U.S. variant. ARS scientists also showed that more recent infestations of fire ants in Australia, China, and Taiwan probably originated from the United States rather than from South America. These subsequent invasions of fire ants may have been facilitated by genetic pre-selection in the United States. This knowledge is valuable because the same biological control agents that are effective in the United States are likely to be helpful in Asia and Australia. Establishment of a complex of biological control agents in the United States and other countries where the fire ant has invaded will likely establish the system of natural controls necessary to reduce the impact of this pest.

Gene silencing, a novel method for mosquito control
Toxicants with new modes of action and high specificity are being investigated for mosquito control. Using gene silencing technology or RNA interference (RNAi), ARS scientists in Gainesville, Florida, have designed molecules that inhibit expression of critical proteins in mosquitoes that results in mortality. We have shown that these molecules can be delivered to adult mosquitoes through the cuticle, with other possible delivery methods under investigation. A new Cooperative Research and Development Agreement with industry will enable large-scale production of RNAi molecules to investigate new carriers and delivery methods to mosquitoes. We are currently awaiting issuance of a patent, which was applied for by the U.S. Department of Agriculture in March 2007. This technology will provide completely safe insecticides based on natural chemicals and easily modified for different purposes and to avoid resistance.

New ways to stop mosquito bites
Insect repellents applied to the skin are widely used to prevent mosquitoes from biting, but they are usually used as a last resort when other forms of control have failed. The promise of highly effective, easy-to-use repellents depends on a precise understanding of how these useful chemicals work. ARS researchers at Beltsville, Maryland, showed that

insect repellents confuse mosquitoes in the manner that they stimulate the organs mosquitoes use to find humans. In the absence of other odors, the repellents activate the organs in a way that does not occur in nature, probably interfering with the ability of the mosquito to find a host. If odors are present that would usually attract the mosquitoes to a host, the repellents prevent detection organs from sensing the chemicals that would normally direct the mosquito to the host. The research demonstrates that repellents can have positive and negative effects on mosquito reception. After we understand how repellents work, we will be able to develop chemicals that work more precisely with the hope that we can manage mosquito behavior more effectively. Products could include more powerful insect repellents, spatial repellents that keep mosquitoes out of a home or backyard, and "herding" chemicals that put mosquito populations into areas where they either cause no harm or where they can be killed.

Mosquito immune response to a pathogenic virus

CuniNPV is a mosquito specific baculovirus that infects mosquito larvae within the genus *Culex*, important vectors of encephalitis viruses worldwide. Mosquito viruses have to overcome insect resistance mechanisms, just as human viruses have to overcome human immune systems. This work demonstrated the presence of a very unusual insect resistance mechanism and the virus' ability to overcome it. Basically, the virus appears to shut down the ability of the mosquito to modify its cellular architecture and the mosquito responds by overproduction of a chemical that regulates the process. ARS scientists in Gainesville, Florida, together with researchers at the University of Florida have investigated mosquito genes that are involved in the pro-apoptotic (programmed cell death) response to viral infection. *Culex* larvae were challenged with CuniNPV and the expression profile of the pro-apoptotic gene mx was measured. There was not a significant increase of mx expression before 8 hr post infection (p.i.) but the level of mx expression continued to increase throughout the infection period and at 48 hr p.i. was about 10 times higher than the uninfected controls. The induction of mx did not result in apoptosis but rather necrosis (degradation of cells) indicating that CuniNPV prevents apoptosis despite the very high level of mx expression. It is possible that CuniNPV utilizes an as yet unknown, but powerful, mechanism to block the apoptotic pathway downstream of mx activation. Identification of this mechanism could have important implications on how other viruses in mosquitoes evade the host immune response, providing innovative new mechanisms for mosquito control.

Realistic assessment of mosquito behavior following exposure to insecticides and repellents

Control efforts against adult mosquitoes primarily consist of aerial spraying and residual treatments, usually using pyrethroids that affect the insects' nervous system. Insect repellents, often containing the active ingredient DEET, are used to reduce the number of bites from populations of mosquitoes that have otherwise escaped other control efforts. Not all mosquitoes receive a lethal dose of insecticide and the effect of sublethal exposure on sensory organs is poorly characterized. ARS scientists in Gainesville, Florida, exposed female *Aedes aegypti*, *Anopheles albimanus* and *Culex quinquefasciatus* to LD25 levels of pyrethroid treatments (the dose necessary to kill only 25% of the mosquitoes). Mosquitoes treated with insecticides tended to fly a less direct route to an attractant and fewer of them responded to the attractant after 24 hours. This suggested that pyrethroids may have a greater impact on disease transmission than their immediate killing impact. Filming the flight behavior of host-seeking female *Aedes aegypti* and *Anopheles* in the presence of pyrethroid and DEET-treated uniforms, it was found that mosquitoes always detected the presence of the blood meals as indicated by their flight towards the blood meal, therefore, DEET did not mask the odors. However, both the DEET and pyrethroid treatment reduced the number of mosquitoes taking blood meals. Mosquitoes attempting to feed through the DEET were repelled and after 10 minutes the majority had stopped trying to feed. In the pyrethroid treatment, mosquitoes tried to feed throughout the entire 20 minute trial and this continued contact with the treated substrates often resulted in 10% mortality of the mosquitoes. Neither repellent nor insecticide created a spatial barrier or prevented mosquitoes from landing and attempting to feed through treated material. This work shows that permethrin and DEET have different effects, though both are used as repellents. Developing new active ingredients that affect mosquitoes in precise ways will lead to new ways to protect people from bites.

Cutting edge genomics applied to stable fly control

Complete sequencing of key arthropod species has formed a library of gene sequences which has been essential in the process of unlocking the identification of genes in many other species of pests. ARS scientists in Kerrville, Texas, have used more streamlined methods of gene identification to find 21 genes associated with host seeking and egg-laying in stable flies, including the first olfactory and taste receptors to be reported for this significant livestock pest. They were also able to find the specific change in a gene that result in resistance to the commonly-used veterinary pesticide, permethrin. Through laboratory selection, University of Florida researchers previously obtained a 15-fold resistance to permethrin in a strain of stable flies. In collaboration with Gainesville, Florida, an ARS scientist at Kerrville, Texas, identified a mutation in the stable fly sodium channel gene that associates with the observed resistant phenotype. Among the many potential products from these discoveries are highly specific molecular pesticides, design of chemicals that alter stable fly behavior, and methods for field detection of insecticide resistance.

Discovery of termite enzyme systems for pest control and bioenergy

There are few molecular target sites for the development of new termite control chemicals. ARS scientists in New Orleans, Louisiana, have cloned two enzymes from the Formosan subterranean termite and expressed them in a bacterial expression system. Incubation of the two enzymes together with cellulose results in digestion of the cellulose to glucose. This will allow us to test a variety of possible inhibitory compounds against the digestive enzymes necessary for the termites as a possible wood preservative or termite control agent. An additional outcome for this research is the possible use of these enzymes for the conversion of biomass into biofuel.