Veterinary, Medical, and Urban Entomology (National Program 104)
Annual Report for FY 2016

The mission of National Program 104 (NP 104) is to improve the protection of humans and livestock from blood-sucking arthropods, and from stinging, or otherwise damaging insects. NP 104 research is divided into three components: (1) Medical entomology for the public and military; (2) Veterinary entomology; and (3) Fire ants and other invasive ant pests. Forty one permanent scientists in 13 projects conduct translational and applied research under these components to mitigate the impact of arthropods such as ticks, mosquitoes, sand flies, stable flies, biting midges, and bed bugs. Non-biting flies such as house flies, filth flies, and New World screwworms are also the targets of this research effort as are invasive ants. The ultimate goal is to protect humans and livestock from these arthropod pests, through the development of safe and effective methods of management and control.

NP 104 research is conducted in ARS laboratories located in six States; these laboratory/units and locations include:

Agroecosystem Management Research Unit, Lincoln, Nebraska;
Arthropod Borne Animal Diseases Research Unit, Manhattan, Kansas;
Biological Control of Pests Research Unit, Stoneville, Mississippi;
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 (2 projects);
Natural Products Utilization Research Unit, Oxford, Mississippi;
Screwworm Research Unit, Kerrville, Texas;
Tick and Biting Fly Research Unit, Kerrville, Texas (4 projects).

The quality and impact of NP 104 research in 2016 was evidenced by the following research-related activities and products:

- 7 new invention disclosure or patent applications;
- 4 patents issued;
  - Patent No. 9,387,921: Self-closing net door for aircraft.
  - Patent No. 9,255,254: Novel Nylanderia Fulva virus.
  - Patent No. 9,408,896: Vaccination of companion animals to elicit a protective immune response against tick infestations and tick-borne pathogen transmission.
  - Patent No. 9,265,818: Genetically modified Babesia parasites expressing protective tick antigens and uses thereof.
- 4 active Cooperative Research and Development Agreements; and
- 15 active Material Transfer and Material Transfer Research Agreements.

These technology transfer efforts include the development of better insecticides and insecticide formulations, traps, and repellents. NP 104 scientists work closely with the

U.S. Environmental Protection Agency (EPA), as subject matter experts on bed bugs, mosquitoes, ticks, and provide input regarding repellent labeling. In addition, NP 104 personnel provide the USDA Animal and Plant Health Inspection Service (APHIS) with direct research support of their agency's Imported Fire Ant Phorid Fly rearing and release program, the Imported Fire Ant Quarantine, Cattle Fever Tick Eradication Program, and Screwworm Eradication Program.

Scientists in NP 104 published 73 papers detailing their research findings in peer-reviewed journals such as Advances in Entomology, Journal of Economic Entomology, Journal of Insect Science, Journal of Medical Entomology, PLoS ONE, Parasitology Research, and Veterinary Parasitology. Research results were also communicated in numerous trade journals that target our customer/stakeholder base.

Internationally, NP 104 scientists participated in research collaborations with scientists in Argentina, Australia, Bolivia, Brazil, Canada, China, Costa Rica, Ecuador, Greece, India, Italy, Japan, Kenya, Mexico, New Zealand, Panama, Paraguay, Peru, Philippines, South Africa, Sweden, Switzerland, Taiwan, Thailand, United Kingdom, and Uruguay. These research collaborations allow access to places where many of our invasive species originated, and also increase the depth of our intellectual capital with original ideas from different perspectives.

Personnel in NP 104

<u>The following scientists in NP 104 received prominent awards in 2016:</u>

Kristina Friesen & David Taylor, Lincoln, Nebraska, received the 2016 National Excellence in Multistate Research Award.

Edward F. Knipling & Raymond C. Bushland, were posthumously honored for their study of the "Sex life of the screwworm fly" at the Library of Congress 5th Annual Golden Goose Award Ceremony.

David Taylor, Lincoln, Nebraska, received the 2016 Lifetime Achievement Award in Veterinary Entomology.

Funding

Fiscal year funding for research conducted under the auspices of NP 104 approached $25 million, of which $7.3 million of these funds were received through extramural agreements. The Deployed War-Fighter Protection (DWFP) Program continues to provide approximately $3 million per year to support research directed at arthropod-borne diseases, and the development of products for protection of military personnel. The DWFP is funded by the Department of Defense, and administered by the Armed Forces Pest Management Board. Some of these funds are also used to support the Aerial Application Technology Research and Insect Control and Cotton Disease Research laboratories in College Station, Texas, the IR-4 Project (minor use pesticide registration) at Rutgers University in New Jersey, and the Navy Entomology Center of Excellence, in Jacksonville, Florida.

Notable Research Accomplishments by Program Components

Component 1: *Medical Entomology for the Public and the Military*

An early warning system for Rift Valley fever (RVF). RVF is a devastating, mosquito-borne disease that affects both livestock and humans. Few opportunities exist to validate or evaluate warnings and control measures in areas where the risk of transmission is high. To better understand, validate, and evaluate the risk for transmission of RVF and other diseases such as Zika and dengue, ARS scientists and several U.S. Government colleagues studied the potential for outbreaks of this disease in Kenya and developed models based on key environmental signals. Alerts produced by the ARS-developed outbreak warning system compelled the government of Kenya to conduct a mass vaccination of domestic livestock, thereby possibly averting a major outbreak of the disease.

Evaluating pesticide application methods to control Zika vectors. The capability of larvicide sprays to penetrate into buildings or through vegetation where mosquitoes may be resting or hiding is not well known. ARS researchers in Gainesville, Florida, in partnership with the Florida Army National Guard, investigated the efficacy of liquid larvicide against mosquitoes that are responsible for the spread of Zika, chikungunya, yellow fever, and dengue viruses. The scientists compared spray applications of a larvicide that targets mosquitoes in a simulated urban environment to mimic conditions in Florida, a hot-arid desert environment as one would find in California, and a dry-season tropical environment typical in Thailand. Results indicated poor penetration into buildings and vegetation in all three environments, even when sprayed at point-blank range. These field trials demonstrate that it may not be possible to effectively control these mosquitoes with traditional methods, but instead, they will require techniques and formulations that that must yet be developed.

Ability of mosquito species to transmit diseases not currently present in the United States. For a very long time, Japanese encephalitis virus (JEV) has been transmitted by mosquitoes in tropical and subtropical regions of Asia. The transmission cycle involves domestic pigs and avian species that serve as amplification hosts; humans are incidental hosts that cannot develop a high-titer viremia sufficient to pass on to mosquitoes. In multiple Asian countries, people bitten by infected mosquitoes can suffer from severe neurological problems. The potential introduction of JEV into North America is a major threat to human and animal health. In this study, ARS researchers from Manhattan, Kansas, collected *Culex* mosquitoes from fields around Valdosta, Georgia, that were shown to be susceptible to JEV infection, suggesting the United States may be at risk of JEV transmission if the virus were to be introduced.

Bed bug defensive secretions attract adult bed bugs. Detection of bed bugs and monitoring for their presence are important first steps in any strategy aimed at controlling these blood-sucking insects, yet there is no monitoring device in widespread use. Using a video tracking system, ARS scientists in Beltsville, Maryland, in conjunction with scientists at the University of

Maryland, demonstrated that chemicals produced by bed bugs that were previously thought to be defensive secretions because they dispersed bed bugs, will actually attract adult male and female bed bugs when released at low levels. This information will be useful for commercial organizations that wish to develop cost-effective, reliable means of monitoring and detecting bed bugs.

Gene silencing technology for mosquito control. There are very few public health pesticides available for controlling medically important vectors such as mosquitoes and sand flies. A novel approach to mosquito control is based on the technology that allows the specific silencing of genes that are critical to survival of the insect species. This technology uses double-stranded RNA (dsRNA) and the process of RNA interference (RNAi) to selectively silence gene products (proteins) that debilitate the mosquito vector and prevent disease transmission. ARS scientists and collaborators in Gainesville, Florida, have demonstrated that targeted silencing of ribosomal proteins RPS6 and RPL26 reduced fecundity more than 88 percent in *Aedes aegypti* mosquitoes for the first oviposition cycle. Reduced fecundity continued through a second oviposition cycle, indicating a lengthy effective period from one treatment. These studies indicate that target selection, dsRNA format, dose, and tissue susceptibility are critical parameters that must be considered in the development of effective RNA-based pesticides to control mosquito vectors. Selectively silencing the production of particular proteins is an effective method for controlling mosquito vectors.

Mosquito bite protection built in to U.S. Marine Corps uniforms. The bite protection of permethrin-treated U.S. military combat uniforms is usually evaluated for 50 wash cycles. This wash cycle end point was chosen on the basis of the expected life time of older, 100 percent cotton combat uniforms. The new combat utility uniforms for the U.S. Marine Corps are composed of 50 percent nylon and 50 percent cotton and last longer than 50 wash cycles. ARS researchers in Gainesville, Florida, conducted bite protection studies of blouse and trouser uniform fabrics washed up to 150 times. The blouses provided 95 percent or better bite protection after 120 wash cycles, whereas the trousers fell below 95 percent after 50 wash cycles. These data establish that uniforms remain intact after 150 wash cycles and for blouses, the treatment is very efficient at preventing bites after 130 washes. This information will be used by the U.S. Marine Corps to better understand the bite protection provided by permethrin-treated uniforms when they are washed up to 150 times.

Mosquito repellent compounds identified in native herb. Sweetgrass is an herb that has been traditionally used by Native Americans as an insect repellent. ARS scientists in Oxford, Mississippi, evaluated extracts from the plant to confirm and determine the basis for the insect repellent claims to discover novel repellents. The sweetgrass extracts did prevent mosquito feeding, and upon further separation of the extracts, scientists identified many repellent components. They determined that phytol and coumarin were responsible for deterring biting by the *Aedes aegypti* mosquito, a vector of diseases such as Zika, yellow fever, and dengue fever. These compounds are known to have mosquito repelling properties and these findings validate the traditional use of sweetgrass as deterrent to mosquito biting.

Component 2: *Veterinary Entomology*

Novel formulation of a vaccine against cattle fever. Cattle fever is a devastating disease transmitted by cattle fever ticks. New technologies are desperately needed to keep the United States free of cattle fever ticks because they are becoming resistant to chemical treatment and new ticks are being brought into the United States on a variety of different animal hosts. The first doses of a new anti-cattle fever tick vaccine were delivered to the Cattle Fever Tick Eradication Program. This resulted from more than 5 years of cooperative research and development among ARS researchers in Kerrville and Edinburg, Texas, and colleagues at several other institutions. This new vaccine formulation is now being used in an integrated vector control program to control the cattle fever tick.

Using integrated pest management technology to control invasive cane. *Arundo* cane is a non-native invasive plant species that is clogging portions of the Rio Grande River and reducing border visibility. ARS researchers in Edinburg, Texas, developed an integrated pest management method to meet the needs of stakeholders along the Rio Grande River. The method integrates mechanized topping of Arundo cane at 3 feet followed by a release of biological control agents that significantly reduce the growth of the cane, thus providing immediate visibility of the international border for law enforcement agencies and, at the same time, long-term suppression of *Arundo* cane. An additional benefit from this practice is that it allows desirable native vegetation to thrive again in the treated area. This integrated pest management process has been transferred to vegetation managers at the U.S. Border Patrol and is being implemented widely on the Texas-Mexico border.

Managing ammonia emissions from screwworm larval rearing media. Mass production and release of sterile screwworms are essential to the successful eradication of screwworms, an insidious and economically important pest in North, Central and South America. The protein-rich diets required for larval screwworms led to high ammonia levels within a mass rearing facility in Panama. Addition of a combination of powder extract of Yucca and potassium permanganate resulted in lower ammonia levels and replaced formaldehyde, which is used as a preservative in the diet. Lower ammonia levels and elimination of formaldehyde in the mass-rearing facility improves conditions for employees and results in a better quality of screwworms, the ultimate product of the facility.

Horn flies can spread *Salmonella*. Insects have been implicated in the spread of microbial pathogens within livestock production systems, and this is important from animal and human health perspectives alike. In cattle, peripheral lymph nodes contaminated with *Salmonella* can be inadvertently processed along with the beef carcass, resulting in tainted beef products. The bacteria may be introduced to cattle by biting arthropods. ARS scientists identified the horn fly as a mechanical vector of *Salmonella* and demonstrated transmission of the bacteria to cattle peripheral lymph nodes due to feeding by horn flies. Improved fly management practices in livestock production systems are possible by using this knowledge to interrupt the horn fly feeding cycle and decrease the likelihood of *Salmonella* in beef.

Novel botanically based biting fly repellent. Flies and other biting insects are annoying and potentially dangerous because of their ability to transmit diseases to humans and livestock. ARS scientists in Lincoln, Nebraska, discovered novel anti-feeding and repellent compounds from a natural product, coconut oil. These compounds provided up to 2 weeks of repellency against biting flies, and 1 week against bed bugs and ticks. The compounds also stop mosquitoes from biting. Efficacy of these compounds is comparable, and in some cases better, than that of DEET, the best biting insect repellent commercially available.

Component 3: *Fire Ants and Other Invasive Pest Ants*

A new kit to identify fire ants. The imported fire ant quarantine has been in place since 1958, regulating the interstate movement of certain commodities in an effort to reduce the spread of imported fire ants. Rapid ant identification at border inspection stations and ports is critical to facilitate trade and commerce. A significant problem is that it is not always possible to identify fire ants because few specimens are collected and it is difficult to identify native and imported ants by physical examination. Researchers with ARS in Gainesville, Florida, and their APHIS colleagues in Biloxi, Mississippi, have developed a field-portable, rapid detection kit to identify imported fire ants. The kit requires no special training or equipment and takes only 10 minutes to confirm whether or not the ants are fire ants. APHIS plans to use the kits at interdiction sites to enforce the quarantine. In addition, regulatory agencies from other countries are interested in adopting the technology.

Improving ant bait formulations. Ant baiting is an efficient and environmentally friendly method of controlling pest ants. Current ant bait products are effective against lipid feeding ant species like fire ants and thus the insecticides used in these baits are oil soluble. However, many invasive pest ants do not feed on oils, and prefer carbohydrate, or sugar solutions. In order to incorporate oil soluble insecticides into a sugar solution, surfactants are usually needed. Unfortunately, surfactants very often deter ant feeding. ARS researchers in Stoneville, Mississippi, found that certain organic solvents, under certain concentrations, did not deter ant feeding. Such solvents can be used in sugar water to increase the solubility of oil soluble insecticides. This has the potential to lead to the development of ant baits effective against the many species of non-lipid feeding invasive ants.