Veterinary, Medical, and Urban Entomology (National Program 104)
Annual Report for FY 2019

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.

The goal of this research program is to conduct fundamental, applied and translational 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.

There are 33 permanent scientists in 11 projects conduct research in ARS laboratories located in six States; these laboratories/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;
Livestock Arthropod Pests Research Unit, Kerrville, Texas (3 projects).

Fiscal year funding for research conducted under the auspices of NP 104 approached $25 million, of which approximately $6 million of these funds were received through extramural agreements. The Deployed War-Fighter Protection (DWFP) Program continues to provide more than $5 million annually to support research directed at detecting and controlling arthropod-borne diseases, and the development of products for protection of deployed military personnel. The DWFP is a Department of Defense program and is administered by the Armed Forces Pest Management Board. ARS receives more than $2.5 million of these funds annually of which a portion directly supports NP 104 research at the Invasive Insect biocontrol and Behavior Laboratory in Beltsville, Maryland and the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida. DWFP funding also supports the Navy Entomology Center of Excellence in Jacksonville, Florida which works in close collaboration with USDA-ARS CMAVE.

The quality and impact of NP 104 research in 2019 was evidenced by the following research-related activities and products:

- 8 new invention disclosure or patent applications;
- 4 patents issued;
  - Patent No. 10,093,925: Lepidopteran Moth Control Using Double-Stranded RNA Constructs.
  - Patent No. 10,093,928: Double-Stranded RNA Constructs to Control Insect Pests
  - Patent No. 10,351,850: Double-Stranded Ribonucleic Acid as Control Against Insects
  - Patent No. 10,363,292: Vaccination with Anti-Tick Antigens to Control Multiple Tick Species and Disease Transmission in White-Tailed Deer and other Host Animals
- 1 new Cooperative Research and Development Agreement;
- 2 new Reimbursable and Trust Agreements;
- 9 new Interagency Agreements; and
- 17 new 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 the Imported Fire Ant Quarantine, Cattle Fever Tick Eradication Program, and Screwworm Eradication Program.

Scientists in NP 104 published 81 papers detailing their research findings in a wide variety of peer-reviewed journals that cover a diverse range of disciplines. The following are select examples:

- Biology and Behavior- Nature Scientific Reports, Genes Genomes Genetics, Frontiers in Ecology and Evolution, PLoS One, Viruses;

- Biochemistry and Chemistry - Chemistry and Biodiversity, Pesticide Biochemistry and Physiology;

- Entomology - American Entomologist, Biocontrol Science and Technology, Environmental Entomology, Insects, International Journal of Acarology, Journal of Economic Entomology, Journal of Vector Ecology, Medical and Veterinary Entomology;

- Medicine/ Public Health - Comparative Immunology Microbiology and Infectious Diseases, International Journal of Environmental Research and Public Health, PLOS Neglected Tropical Diseases

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, Brazil, Canada, China, Costa Rica, Ecuador, Egypt, France, French Polynesia, Greece, Israel, Italy, Kenya, Mexico, New Zealand, Panama, Sweden, Thailand, Ukraine, United Kingdom and Vietnam. 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

New scientists in NP 104 2019:

Dr. Glen Scoles, Research Entomologist, formerly with the Animal Disease Research Unit in Pullman, Washington, joined Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Dr. Alex Arp, Research Geneticist (Insects), joined Knipling-Buschland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Dr. Kyle Harrison, Research Entomologist, joined Agroecosystem Management Research Unit, Lincoln, Nebraska.

The following scientist retired:

Dr. Felicito (Felix) Guerrero, Research Physiologist, Knipling-Buschland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

The distinguished record of Dr. Guerrero is recognized world-wide and he will be missed at NP 104.

Notable Research Accomplishments by Program Components

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

Easily deployed spatial repellent provides protection from disease vectors. Standard pesticide sprays can reduce mosquito and biting fly attacks. However, they also contribute to insecticide resistance, which undermines their long-term utility. ARS scientists in Gainesville, Florida, with collaborators determined that the spatial repellent transfluthrin applied to strips of camouflage netting and geotextiles, used ubiquitously by the U.S. military, can be easily transported and attached to perimeters and structures to create a rapid shelter from biting and disease-vectoring insects. Significant reductions of incursions by mosquitoes, sand flies, and other biting flies were documented in protected perimeters across four ecologically distinct and militarily relevant environments. Thus, deployment of vector protection occurred without having to wait for

intervention by mosquito and vector control units. In addition, the spatial repellent protected the perimeters without causing death of the targeted pests. By not killing the insects, the development of insect resistance is impeded by allowing insecticide-susceptible insects to dilute populations of insecticide-resistant pests.

Mosquito repellency of pineapple weed compounds. Pineapple weed has documented use as an insect repellent by various indigenous peoples of North America. This investigation by ARS scientists at Oxford, Mississippi, was conducted to identify chemical constituents responsible for any insect repelling effect and evaluated the practice via a mosquito-biting-deterrent test. Essential oil from dried pineapple weed deterred biting by mosquitoes as effectively as diethyltoluamide (DEET), which is used in retail mosquito repellent products. The most active compound isolated from pineapple weed, alpha-terpineol, provided complete protection similar to DEET. In addition, another compound in pineapple weed, neryl isovalerate, was for the first time discovered to have mosquito repellent properties. This research provides scientific evidence that validates the traditional use of pineapple weed as a biting-insect deterrent and reveals a potential natural alternative to widely used synthetic insect repellents.

Volatile identified from bed bug eggs. New strategies to detect bed bugs are always in demand for both the public and companies involved in detecting these blood-sucking insect pests. Canines trained to detect bed bugs have been used for about a decade and ARS scientists in Beltsville, Maryland, identified the compounds produced by bed bug nymphs and adults that alert the trained dog to their presence. A study was undertaken to determine whether the chemical compound in bed bug eggs was similar or identical to the chemical produced by nymphs and adults. Surprisingly, the chemical identified that emanated from bed bug eggs was different. Furthermore, neither of the compounds produced by nymphal and adult bed bugs could be detected in the egg samples. This information will be useful to industry personnel attempting to develop new detection methods for bed bugs.

Component 2: *Veterinary Entomology*

Reduction of an invasive weed in the cattle fever tick quarantine zone. The giant reed is an invasive weed in the cattle fever tick Permanent Quarantine Zone, where it clogs portions of the Rio Grande River and reduces border visibility. Two biological control agents of the giant reed, the arundo wasp and the arundo scale, were released in 2009 and 2010, respectively. Nine years after the release of the scale, ARS scientists in Kerrville, Texas, documented 55 percent less above-ground biomass of stands of the giant reed in areas where both the arundo wasp and scale were used as a biocontrol agent compared with areas where the wasp alone was used. Despite its low dispersal rate, the impact of the arundo scale as a biological control agent was augmented when used together with the wasp. Reducing stands of giant reed decreases the ideal habitat for southern cattle fever ticks, which helps maintain the cattle fever tick quarantine.

Salivary proteins of biting midges associated with virus transmission in livestock. Until this research occurred, the extreme efficiency with which biting flies (midges) can transmit some viruses was not clearly understood. ARS scientists in Manhattan, Kansas, discovered that when virus-infected midges bite seeking blood, along with the virus they deposit 45 proteins in their saliva,

which are critical for successful acquisition of a bloodmeal. A mouse model showed these salivary proteins promoted rapid infection and systemic dissemination of midge-transmitted viruses via the lymph system. Additionally, saliva-induced blood vessel dilation encourages virus replication and dissemination via the circulatory system. This research advances our understanding of the complex myriad of proteins in midge saliva and provides insights into their functional role in blood feeding, virus transmission, and viral disease pathogenesis. This fundamental research guides the development of methods to impede virus transmission in livestock.

Translational research advances tick-borne disease diagnostics. Most tick-borne diseases can be transmitted by infected ticks through their bite from animals to humans. Enhanced pathogen detection is needed to improve the diagnoses of these diseases. The TickPath Layerplex is an innovative molecular assay that detects several tick-borne pathogens from ticks, animals, or humans. It is being patented by ARS scientists in Kerrville, Texas, and collaborators at Texas A&M University–College Station. The TickPath Layerplex detects several groups of tick-borne pathogens in a sample, distinguishes the type of tick-borne pathogen, and thereby guides decisions for rapid and appropriate treatments. This biotechnology aids in the diagnosis of human and animal tick-borne diseases.

Surveillance of pesticide resistance in southern cattle fever tick (SCFT). The SCFT may be the most economically important tick species due to its negative impacts on beef and milk production. Keeping the United States free from SCFT depends on the quarantine of infested premises and the systematic treatment of cattle with pesticides to suppress tick populations. The development of pesticide-resistant SCFT populations is becoming a problem and is associated with the intense use of pesticides to keep livestock free from SCFT. A rapid molecular test was developed by ARS scientists in Kerrville, Texas, to simultaneously detect different mutations in the SCFT genome associated with resistance to pyrethroids, a pesticide commonly used against ticks. Results from the test were used to evaluate the temporal epidemiology of pyrethroid resistance and SCFT outbreaks in the United States. Rapid surveillance for pesticide resistance is critical in the design of strategies to prevent the development of pesticide resistant SCFT populations and to promote sustainable SCFT management.

Development and validation of CRISPR-Cas9 for gene knockout in screwworms. The continual release of sterile screwworms is essential to the successful eradication of this insidious and economically important pest of livestock and wildlife in Central and South America. ARS scientists at Kerrville, Texas, collaborated with scientists at the North Carolina State University and University of Campinas in Brazil to develop and validate methods for using the genome editing technology CRISPR-Cas9 in the screwworm. CRISPR-Cas9 can be used to selectively knock out a target gene and insert transgenes in specific locations in the genome. This technology is a key tool in developing gene drive strains and can be used to understand gene function. The technique was verified by knocking out genes for body color, olfaction, and sex determination. This method may be used to advance the sterile screwworm release program with gene drives or female-to-male transformation systems.

Coconut oil-based repellent formulations for protection from biting insects. Flies and other biting insects are annoying and potentially dangerous, and cause suffering and transmit diseases among humans and livestock. ARS scientists in Lincoln, Nebraska, and Peoria, Illinois, developed a novel,

starch-pectin water-based formulation of coconut fatty acids for use on livestock and companion animals. This formulation repels biting flies for up to 4 days in pasture settings. Potentially, coconut fatty acids could be used in sunscreen-based and lavender oil-based formulations and lotions to effectively repel mosquitoes and ticks from humans and animals.

Pesticide resistance not detected in the invasive Asian longhorned tick. The invasive Asian longhorned tick can vector serious diseases in humans and animals. First discovered in the United States in 2017, its presence has been confirmed in 12 States by 2019. To determine the susceptibility of this tick species to pesticides commonly used in tick control (specifically pyrethroids), ARS scientists in Kerrville, Texas, and cooperators at Rutgers University, characterized the genes associated with pyrethroid resistance. No mutations previously associated with pyrethroid resistance were detected in tick samples from New Jersey. This was the first characterization of a gene in the Asian longhorned tick associated with pesticide resistance and aids in the selection of appropriate tick treatments.

Alternative treatment for southern cattle fever tick-infested deer hides. Hides from hunted white-tailed deer are systematically inspected and treated with pesticides before they leave areas in south Texas known to be at risk of infestation by southern cattle fever ticks (SCFT). This invasive tick is considered to be the most economically important external parasite of livestock worldwide. Safer pesticides are needed to treat deer hides infested with ticks, especially the SCFT. Laboratory experiments by ARS scientists in Kerrville, Texas, showed that a commercial product containing a mixture of essential oils killed 100 percent of the immature SCFT, reduced female fertility by 94 percent, and killed 98 percent of the fully engorged females. Thus, the essential oil product was an effective and safer deer hide treatment for SCFT.

House fly larvae change the microbial community in manure. House fly larvae develop in microbe-rich cattle manure. While the larvae feed on the microbes, little is known about the influence that larval grazing has on manure bacterial communities over time. ARS scientists in Manhattan, Kansas, collaborated with Kansas State University colleagues to compare the bacterial community in manure with and without house fly larvae. By analyzing DNA molecular sequences of bacterial samples collected from the manure, the number of different bacteria species (richness) and the number of bacteria per species (abundance) were both significantly lower in manure that contained larvae. In addition, abundances of the bacterial phyla Bacteroidetes and Proteobacteria significantly increased, whereas abundance of the bacteria in phylum Firmicutes decreased after larvae developed for 10 days in the manure. These results demonstrated that house fly larval feeding substantially alters bacterial community diversity in cattle manure. Further analyses of these data will examine how larvae influenced the presence of pathogenic bacteria in order to understand whether house fly presence affects the risk of manure-associated pathogen transmission to humans.

Habitat shapes the bacterial communities in the gut of female house flies. Adult house flies feed and breed in a wide range of microbe-rich habitats, and they harbor and transmit bacteria including many human and animal pathogens. ARS scientists from Manhattan, Kansas, with Kansas State University colleagues, characterized the bacterial communities in guts of female house flies collected from agricultural (beef cattle feedlot), urban (business area dumpsters), and mixed (business located near agriculture) environments in Kansas. Based on next-generation sequencing of the bacterial 16S rRNA gene, the taxonomic types and specific species of bacteria associated

with flies differed between flies collected from the different sites. Potential human pathogenic bacteria were associated with flies from the mixed environment; and feces-associated bacteria were found in flies from both the agricultural and urban environments. Results indicated that the house fly gut harbors complex bacterial communities, including potential human and animal pathogens, and that the bacterial communities are strongly influenced by the fly habitat. Understanding the factors affecting the risk of house flies vectoring pathogens to humans and animals will guide the development of mitigation strategies.

Artificial sweeteners offer an alternative tool for fly control. Although their mode of action is not known, the artificial sweeteners xylitol and erythritol are toxic to several species of adult flies. In this study, an ARS researcher in Gainesville, Florida, and a colleague at Northern Illinois University evaluated the effect of these sweeteners on larvae of house flies and stable flies. Larvae of both species were more sensitive to erythritol than xylitol, and stable flies were more sensitive than house flies. Adult flies could not distinguish between untreated larval media and media treated with the sweeteners, and they readily laid eggs on both types. As a result, larvae that emerge from eggs are exposed to treated media. These sweeteners appear to have potential as an inexpensive way to control flies without using conventional insecticides.

House fly susceptibility to low-cost essential oils. House flies are resistant to every known fly control insecticide, so new fly control tools are critically needed. Essential oils of vetiver, cinnamon, lavender, and sunflower were evaluated for insecticidal and repellent properties by an ARS researcher in Gainesville, Florida, and an Egyptian colleague. All oils caused 94 to 100 percent mortality of fly larvae and killed 100 percent of exposed adults. Vetiver and cinnamon oils repelled 84 and 78 percent of larvae, respectively, but not adult flies. Adults were repelled by neem oil and *p*-methane-3,8-diol (PMD). Based on efficacy and cost, cinnamon oil has the most potential for further development. It is classified by the Environmental Protection Agency as sufficiently safe, which precludes registration as a pesticide. Thus, new products containing this oil could be developed more easily and with few regulatory challenges.

High temperatures give house flies an advantage over their natural enemies. House flies are difficult to control and are an important pest of humans and animals. Today, fly control is conducted under hotter conditions because of climate changes, but most management strategies were designed for regions with moderate temperatures. Hot temperatures could affect the balance between the fly and wasp parasites that kill the fly. An ARS researcher in Gainesville, Florida, and Israeli colleagues compared the heat tolerance of fly and wasp populations in the United States. Only one wasp species, *Muscidifurax zaraptor*, was as heat-tolerant as the flies. Results show that *M. zaraptor* is the most effective wasp parasite for fly control under hot conditions. More heat-tolerant wasp populations might be discovered with further exploration.

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

Improved diagnostic kit for all quarantined fire ant species in commercial development. The red imported fire ant and the black imported fire ant are invasive species that cost more than $6 billion annually in damage and control measures in the United States. The Federal imported fire ant quarantine program requires commodities to be free of both species before leaving quarantine. ARS

scientists in Gainesville, Florida, with Animal and Plant Health Inspection Service scientists in Biloxi, Mississippi, developed a simple-to-use and portable identification kit (analogous to a home pregnancy test) that can identify both fire ant species from all other ants in a single, 10-minute test. The speed of the test curtails extended delays at inspection stations by eliminating the need to send off samples for identification. Being able to distinguish the black imported fire ant is an important improvement over the previous kit (InvictDetect) the scientists developed, which detected only the red species. The improved kit provides a new tool for regulatory agencies in the United States and other countries to enforce quarantine protocols that limit the spread of one or both invasive ant species. The license for this new technology has been acquired by Agdia Inc. and is currently being developed for commercial distribution.

Nine new viruses discovered in fire ants from South America. Imported fire ants were accidently introduced into the United States in the 1930s and currently infest more than 365 million acres. They cause more than $6 billion in damage and control costs annually in livestock and agricultural production, buildings and landscapes, and pose a serious threat to human health. ARS researchers in Gainesville, Florida, have discovered nine new viruses in fire ants from South America, two of which exhibit unique genome structures, which suggests that they may be a new category of viruses. The nine viruses have not been detected in the United States and potentially could be released into the United States fire ant population as biological control agents. Biological control agents spread naturally and are self-sustaining. When biocontrol agents are effective at reducing populations, they are considered to be the most efficient and environmentally compatible control method for well-established invasive pests such as fire ants.

New formulations for controlling fire ants in potting soil. The long distance movement of fire ants often occurs through the transport of infested potted plants. To slow or prevent the spread of fire ants, a Federal quarantine requires potted plants to be treated with a significant amount of synthetic insecticides before they can be moved from quarantined areas. A major concern for such treatment is the potential detrimental effect of insecticides to the environment. ARS scientists in Stoneville, Mississippi, determined that a natural insecticidal formulation using hexyl benzoate controlled fire ants in potting soil for 60 days. This formulation has the potential to be used as an alternative to synthetic insecticides in quarantine treatments. It also was found that fire ants killed nestmate larvae and pupae that received low concentrations of the formulation. This discovery may open new avenues of study on the mechanism of how fire ants recognize members of their colony.

The male fire ant transfers more than sperm to the female. Within a fire ant colony, the mother queen inhibits reproductive development in her winged female offspring (unmated queens) with a pheromone; however, after mating the winged females need to very quickly lay eggs to start their new colonies. ARS scientists in Gainesville, Florida, discovered that male fire ants transfer compounds to the winged females during mating that rapidly initiate egg production and other biological characteristics of a mother queen. This discovery shows how male fire ants help winged females overcome the effects of their mother's pheromone. The discovery reveals potential pathways to disrupt fire ant reproduction, which can lead to novel control methods for this invasive species that causes more than $6 billion in annual damage and control costs in the United States.

Identification of fire ant attractants. Bait technology is commonly used in the control of fire ants, one of the world's worst invasive species. To improve the effectiveness of baits, several new fire

ant attractants were identified from different sources by ARS scientists in Stoneville, Mississippi, and chemists at the University of Mississippi and Zhejiang University in China. Three compounds from ylang-ylang oil, an essential oil extracted from the fragrant yellow flowers of the tree, *Cananga odorata*, and one novel compound from fire ant venom were identified as fire ant attractants. These compounds may potentially be used to enhance the attraction of baits to fire ants and thereby improve bait performance.