Veterinary, Medical, and Urban Entomology (National Program 104)
Annual Report for FY 2021

The mission of National Program 104 (NP 104) is to eliminate arthropod vectors and the diseases that they transmit to livestock, humans, and other animals and to nullify their economic impact. NP 104 research is divided into three components: (1) Veterinary Entomology; (2) Medical Entomology; and (3) Fire Ants and other Invasive Ants.

Approach: The mission will be accomplished through research to develop novel and/or improved risk assessment, surveillance, control, and monitoring tools for arthropods and arthropod-borne diseases of veterinary, medical, and urban importance.

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, and biting midges. 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 13 projects that conduct research in ARS laboratories located in seven 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
- Crop Bioprotection Research Unit, Peoria, Illinois
- 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 (2 projects)
- Cattle Fever Tick Research Unit, Kerrville, Texas
- Veterinary Pest Genetics Research Unit, Kerrville, Texas.

Fiscal year funding for research conducted under the auspices of NP 104 approached $25 million, of which approximately $1 million of these funds were received through extramural agreements. The Deployed War-Fighter Protection (DWFP) Program provided approximately $900,000 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 received approximately 25% of the total FY2020 allocation from this program. The funding directly supports NP 104 research at 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 2021 was evidenced by the following research-related activities and products:

- 6 new invention disclosure or patent applications;
- 4 new Cooperative Research and Development Agreements;
- 11 new Interagency Agreements; and
- 4 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 mosquitoes and 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 115 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 - Scientific Reports, Journal of Vector Ecology, Advances in Entomology, BMC Biology, Brazilian Journal of Veterinary Parasitology, Data in Brief, Ecology Letters, Entomologia Experimentalis et Applicata, Functional Ecology, Insect Science, Insects, Journal of Agricultural and Urban Entomology, Journal of Animal Ecology, Journal of Bangladesh Agricultural University, Journal of Economic Entomology, Journal of Fish and Wildlife Management, Journal of Insect Science, Journal of Pest Science, Pest Management Science, Scientific Reports, South African Journal of Botany, Southwestern Entomologist, Subtropical Agriculture and Environments, The Journal of Visualized Experiments (JoVE), Veterinary Parasitology, Wetlands Ecology and Management, Zootaxa.

- Biochemistry and Chemistry - Journal of Agricultural and Food Chemistry, Insect Science; BMC Genetics, BMC Genomics, Chemosphere, Frontiers in Cellular and Infection Microbiology, Molecules, Natural Product Communications, Phytofrontiers, Toxicon.

- Medicine/ Public Health - Frontiers in Cellular and Infection Microbiology, Journal of the Florida Mosquito Control Association, Florida Entomologist, Foodborne Pathogens and Disease, Journal of Agricultural and Food Chemistry, Journal of Invertebrate Pathology, Journal of Medical Entomology, Journal of the American Mosquito Control Association, Journal of the Florida Mosquito Control Association, Journal of Vector Ecology, Medical Entomology, Parasites & Vectors, Pathogens, Ticks and Tick Borne Diseases, Vector-Borne and Zoonotic Diseases, Virology Journal, Viruses.

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, Ecuador, France, Greece, Kenya, New Zealand, Panama, South Korea, United

Kingdom and Vietnam. These research collaborations allow access to places where many of our invasive species originated, and also increases the depth of our intellectual capital with original ideas from different perspectives. We leveraged the resources at our Overseas Biological Control Laboratories in Argentina, Australia, France, and Greece to make sustained progress in the search for new biocontrol agents and test new control technologies against local pest organisms.

Personnel in NP 104

The following scientist in NP 104 received a prominent award in 2021:

Dr. Jerry Zhu was selected as a recipient for the Office of Technology Transfer (OTT) Pilot Initiative and received a 2021 USDA OTT Research Initiative Award.

Notable Research Accomplishments by Program Components

Component 1: Medical Entomology for the Public and the Military

Novel spatial insect repellent dispensing devices to protect military troops. ARS scientists in Gainesville, Florida, treated bootlaces with various formulations of spatial insect repellents and tested them under laboratory and semi-field conditions. The treated bootlaces provided effective repellency over 3 meters (approximately 9 feet) in all directions against the yellow fever mosquito, *Aedes aegypti*, for more than 3 months. Additionally, novel tent entrance devices were designed and evaluated under semi-field conditions and provided significant spatial repellency against four mosquito species including three genera that carry disease. The yellow fever mosquito spreads numerous human diseases (dengue fever, chikungunya, Zika fever, Mayaro, and yellow fever) and these repelling devices will help protect combat forces deployed to environments where these mosquitos thrive; they could also protect civilians, if they are marketed for the public.

House flies can harbor SARS-CoV-2 but do not transmit infectious virus. The SARS-CoV-2 virus, which is responsible for the COVID-19 pandemic, is highly contagious and is usually transmitted via the respiratory route or after contact with items contaminated by infected persons. ARS scientists in Manhattan, Kansas, and Kansas State University collaborators evaluated whether house flies could acquire and transmit the SARS-CoV-2 virus. Flies were exposed to SARS-CoV-2-spiked culture media or 10 percent milk substrates and tested for the virus at either 4 or 24 hours after exposure. All flies exposed to the SARS-CoV-2- inoculated media or milk were carrying viral RNA at 4 hours and 24 hours post-exposure. However, the infectious virus was detected only from the flies exposed to virus-spiked milk. In a second experiment, flies exposed to SARS-CoV-2 for 24 hours were transferred to a clean container, and no infectious virus was recovered either 4 hours or 24 hours after exposure. These results indicate that in laboratory settings, house flies can acquire and harbor infectious SARS-CoV-2 for up to 24 hours post-exposure but they do not transmit the infectious virus. While house flies likely do not play an important role in SARS-CoV-2 transmission, field-trapped house flies could potentially be used for virus surveillance.

Development of coconut oil fatty acids and derivatives as an insect repellent. ARS researchers in Lincoln, Nebraska, and Peoria, Illinois, recently discovered free fatty acids and their derivatives associated with coconut oil can act as strong spatial and contact repellents against various blood-sucking insects, including mosquitoes, ticks, bedbugs, biting flies, and other nuisance pests. The repellent effectiveness of coconut oil fatty acids and derivatives was demonstrated as equivalent to the gold repellent standard "N, N-Diethyl-meta-toluamide (DEET)"; in addition, coconut oil fatty acids and derivatives provide repellency for more than a week. Working together with several small U.S. business companies, ARS scientists are developing commercial products, including repellent-impregnated textiles, to prevent mosquito bites and associated disease transmission.

Artificial sweeteners are harmful to house flies. House fly control is a global problem because of high levels of insecticide resistance. Recent research has demonstrated that flies are killed when they feed on the artificial sweeteners erythritol and xylitol, which are safe for humans and the environment. The reason for their toxicity to insects had not yet been determined. Scientists at Northern Illinois University worked with ARS scientists in Gainesville, Florida, and Manhattan, Kansas, to evaluate several mechanisms designed to clarify how artificial sweeteners induce mortality in house. Results showed that flies consuming artificial sweeteners die from complications caused by excessive regurgitation. This work supports the continued evaluation of safe materials as tools for house fly control where insecticides are no longer effective, or for use in organic production systems.

Component 2: Veterinary Entomology

Attractant-impregnated adhesive stable fly tape. Stable flies are one of the most important arthropod pests of livestock. Stable flies reduce cattle weight gain and milk production which leads to an annual economic loss of more than $2 billion to the U.S. cattle industry. ARS scientists in Lincoln, Nebraska, have identified novel attractant compounds that can be used with mass trapping techniques to reduce stable fly attacks on cattle. These attractants have been developed with adhesive technologies for stable fly control in feedlots to help cattle producers reduce stable fly infestation levels. A U.S. patent and an international patent application have been filed. A developed prototype product has been tested in the field resulting in improved protection of cattle against biting flies and an 80 percent reduction in cattle stress.

Connecting genes to function in the stable fly. The stable fly is a blood-feeding pest of economic significance to U.S. cattle producers, reducing cattle productivity by an estimated $2 billion annually. Management of this pest is challenging, and novel methods of targeting stable flies are needed to enhance a producer's ability to suppress fly populations. ARS researchers in Kerrville, Texas, and Manhattan, Kansas, collaborated with scientists from 12 U.S. and three foreign universities to sequence and describe the genome of the stable fly. The team identified gene families involved in stable fly olfaction and vision, blood-feeding, reproduction, and metabolism of pesticide compounds. This important resource is being used to identify and target pathways that are critical to stable fly biology with the goal of developing unique strategies to reduce the burden of these flies on livestock production settings.

Methodology to produce large numbers of irradiated *Aedes aegypti males.* *Aedes aegypti*, commonly known as the yellow fever mosquito, spreads dengue fever, chikungunya, Zika fever, Mayaro, and yellow fever viruses. ARS scientists in Gainesville, Florida, developed a novel methodology to produce tens of thousands of irradiated and sterilized male *Aedes aegypti* mosquitoes for use by mosquito control agencies for release in Sterile Insect Technique (SIT) programs. Male mosquitos do not feed on blood but mate with fertile females who will then lay infertile eggs. These standardized techniques were published and should facilitate the production of irradiated males required for mosquito SIT programs, greatly reducing the need for pesticide use and protecting people from these deadly viruses.

United States populations of the invasive longhorned tick can transmit cattle Theileria. The invasive longhorned tick (*Haemaphysalis longicornis*) has spread rapidly in the eastern United States since its initial report in 2017. In its native range, the longhorned tick is the vector of Oriental Thileriosis which is an economically significant tick-borne disease of cattle caused by *Theileria orientalis*. Persistently infected cattle occur sporadically in the United States, but transmission and subsequent disease do not occur without a vector. ARS scientists in Pullman, Washington, and Beltsville, Maryland, infected cattle with an isolate of *T. orientalis* collected from a Virginia farm and transmitted it via tick-borne transmission to uninfected calves to demonstrate that this invasive tick can effectively transmit the disease. Transmission of *T. orientalis* by longhorned ticks represents a significant threat to the U.S. cattle population. The westward spread of longhorned ticks into areas where persistently infected cattle are present may lead to disease outbreaks and result in severe economic burdens on cattle producers. These results provide valuable information for U.S. cattle producers and reinforce the need for continued surveillance and enhanced control measures for this invasive tick.

Remotely operated nematode sprayers provide non-chemical control of cattle fever ticks. Cattle fever ticks (CFT) threaten U.S. animal agriculture because they transmit the microbes that cause bovine babesiosis, a disease that causes rapid death in cattle. In south Texas, wildlife such as white-tailed deer and nilgai antelope serve as alternative hosts for CFT, complicating efforts to eradicate the ticks. A novel technology to treat wildlife infested with cattle fever ticks with parasitic microscopic roundworms or nematodes was successfully tested and shown to be effective. ARS scientists in Edinburg, Texas, worked closely with the Animal and Plant Health Inspection Service-Veterinary Services and ranchers in South Texas to conduct large-scale field tests of a nematode sprayer to eradicate CFT on free-ranging nilgai antelope. More than 100 sprayers were deployed to apply nematodes (Nemasys-R, BASF Co.) across more than 5,000 acres as the nilgai moved through fence crossings. Treated nilgai were found to be infested with significantly lower numbers of cattle fever ticks than non-treated nilgai.

Component 3: Fire Ants and Other Invasive Pest Ants

Invasive ant queens are fertile year-round. The tawny crazy ant is an invasive ant that is spreading in the southern United States. The control of invasive ants requires an understanding of their biology to develop efficient methods of control, such as strategically applying ant baits to eliminate queen ants which are vital to the survival of ant colonies. Previous research indicated that groups of tawny crazy ant queens congregated within nests during the winter, but eggs and immature ants were not being produced. However, monthly examinations of tawny crazy ant queens by scientists in Gainesville, Florida, determined that the queens' ovaries contained eggs year-round and over 80 percent of the queens were mated. These results indicated that despite having mature eggs, egg laying was not occurring, perhaps because there is less foraging for food by the colony and limited feeding by queens during the winter. This suggested that instead of applying baits in the winter to target queens consolidated within nests, an alternative strategy of applying baits in the spring when egg laying starts, colonies are actively foraging, and before colonies split and spread in the summer, may result in better control of this invasive ant.

Insect food webs and invasive ant diets in the U.S. Corn Belt. The rapid increase in bioenergy crops is one of the largest global trends in land use, driven by the need for alternate fuels and to slow climate change. The production of fuel ethanol is now a dominant factor in farming landscapes in the Midwestern United States. Corn Belt. Scientists from Gainesville, Florida, Brookings, South Dakota, and Michigan State University investigated how plant diversity in bioenergy croplands in this region impacted insect food webs and the diets of one of the oldest and most widespread invasive ants in the United States, the pavement ant. They found that invasive pavement ants adjusted their diets based on which biofuel crop they inhabited, feeding on a mix of plant and animal prey in species-rich prairie and switchgrass fields, but preying entirely on other insects in species-poor corn fields. When examining how energy flowed through entire insect food webs, they found that food webs in corn fields were simpler and likely more prone to fluctuations than those in diverse native perennial biofuel crops like fields of switchgrass and restored prairie. The results highlight that by supporting more complex species interactions, restoring farmlands to native perennial biofuel crops can help diversify and stabilize agricultural food webs.