Veterinary, Medical, and Urban Entomology (National Program 104)
Annual Report for FY 2020

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, 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 32 permanent scientists in 10 projects that 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 (2 projects).

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 an additional $1.1 million 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 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 2020 was evidenced by the following research-related activities and products:

- 8 new invention disclosure or patent applications;
- 1 patent issued;
  - Patent No. 10,568,320: Biologically-Based Control Methods for Insect Pests
- 4 new Cooperative Research and Development Agreements;
- 5 new Reimbursable and Trust Agreements;
- 3 new Interagency Agreements; and
- 12 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, 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 79 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, Genes Genomes Genetics, PLoS One, Journal of Vector Ecology, Journal of Apicultural Research, Journal of Wildlife Diseases, Ecosphere;

- Biochemistry and Chemistry - Archives of Industrial Hygiene and Toxicology, Biomolecules, Journal of Agricultural and Food Chemistry, G3, Genes/Genomes/Genetics;Entomology - Biocontrol Science and Technology, Environmental Entomology, Insects, Journal of Vector Ecology, Medical and Veterinary Entomology, Annals of the Entomological Society of America, Insect Science;

- Medicine/ Public Health - Frontiers in Cellular and Infection Microbiology, Journal of Infectious Diseases and Therapy, Journal of School Health, Istanbul Journal of Pharmacy, Journal of Medical Microbiology, Journal of the American Mosquito Control Association, Journal of the Florida Mosquito Control Association

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, French Polynesia, Greece, Israel, Italy, Kazakhstan, Kenya, Netherlands, New Zealand, Panama, South Korea, Sweden, Thailand, 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

<u>New scientists in NP 104 2020:</u>

Dr. Robert Miller, National Program Leader, formally from the Cattle Fever Tick Research Laboratory, Edinburg, Texas joined the Office of National Programs in Beltsville, Maryland.

Dr. Kelly Harrison, Post-Doctoral Researcher, joined the Agroecosystem Management Research Unit in Lincoln, Nebraska

Dr. Bethany McGregor, Research Entomologist, joined the Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas

Dr. Alden Estep III, Entomologist, joined the Mosquito and Fly Research Unit in Gainesville, Florida.

Dr. Edmund Norris, Entomologist, joined the Mosquito and Fly Research Unit in Gainesville, Florida.

<u>The following scientist retired:</u>

Dr. David Taylor, Research Entomologist, USDA-ARS Agroecosystem Management Research Unit, Lincoln, NE
The distinguished record of Dr. Taylor is recognized world-wide and he will be missed at NP 104.

<u>The following scientists in NP 104 received prominent awards in 2020:</u>

Dr. Jerry Zhu, was elected as the President of the International Society of Chemical Ecology. He was also awarded the 2020 OECD Fellowship from the Co-Operative Research Programme: Sustainable Agricultural and Food Systems to conduct research entitled "Development of novel technologies in integrated biting fly management via understanding physiological mechanisms of spatial and contact repellency."

<u>*Notable Research Accomplishments by Program Components*</u>

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

<u>Treated military uniform compromised by pesticide-resistant mosquitoes.</u> Military camouflage uniforms are routinely treated with permethrin to repel disease-carrying mosquitoes. This protects the military service members while they operate in environments where mosquito-vectored diseases are endemic. ARS researchers in Gainesville, FL, and U.S. Department of Defense collaborating scientists completed and published a study clearly demonstrating that treated military uniform efficacy may be completely ineffective at preventing bites by pyrethroid-resistant strains of *Aedes aegypti*, a vector of numerous human diseases. Fortunately, this study also showed that the protection provided by N,N-Diethyl-meta-toluamide (DEET)-based repellents was unaffected by resistance to pyrethroids. This information will aid in the risk assessment of different operational environments and lead to changes in

procedures and chemicals used to protect at-risk military members operating in areas where pyrethoid-resistant mosquitoes exist.

Discovery of synergists for topical and spatial repellents that circumvent pesticide resistant mosquitoes. Pyrethroids are one of the most commonly used classes of insecticides. The acids of three common pyrethroids were shown to be nontoxic to mosquitoes. ARS researchers in Gainesville, FL, along with partners at the University of Florida, discovered these pyrethroid acids have significant synergistic spatial repellent activity. Synergists are compounds that, when added to a pesticide or repellent, greatly increase the efficacy or repellency over the pesticide or repellent alone. The repellency measured was greater than the topical repellent N,N-Diethyl-meta-toluamide (DEET), was often better than the repellency of the parent pyrethroids and showed little cross resistance in a pyrethroid-resistant Puerto Rico strain of mosquitoes. Most important, synergism caused by the pyrethroid acids, when combined with several repellent compounds, increased protection of human arms from biting mosquitoes. Using these synergists may increase bite protection for existing and novel compounds, including protection against resistant mosquitoes that can transmit diseases such as dengue, Zika, chikungunya, and yellow fever.

House flies collected in agricultural settings carry antimicrobial-resistant bacteria. House flies are primary pests of confined livestock operations such as dairies, and due to their close associations with humans, are nuisance pests in domestic settings. Adult flies frequent microbe-rich substrates such as garbage dumpsters and animal manure, where they encounter and ingest bacteria during feeding and reproduction. To assess human and animal health risks, bacteria were enumerated from whole flies, identified to species, and tested for susceptibility to 14 antimicrobials. Both male and female flies carried antimicrobial-resistant (AMR) bacteria: 36 of 38 isolates (95 percent) were resistant to more than one antimicrobial, 33 (87 percent) were multidrug-resistant (MDR); and 24 isolates were resistant to more than 4 antimicrobials. Remarkably, some isolates were resistant to up to 8 types of antimicrobial drugs used in human and animal medicine. Bacterial isolates included both commensal bacteria such as coliforms and pathogenic species of *Providencia*, *Proteus, Serratia* and *Klebsiella*. While AMR carriage by commensal bacteria may not pose a direct threat, these microbes carrying AMR genes can serve as a source of resistance that can be transferred to other bacterial pathogens. Furthermore, house flies have been shown to facilitate this type of lateral gene transfer among organisms which can occur in the gut of the fly. These results suggest that flies may play a role in harboring and disseminating bacteria, including AMR and MDR strains and potential pathogens that pose a risk to both human and animal health. The results add to the growing evidence implicating flies as major players in disease ecology, epidemiology, and possibly the dispersal of AMR genes. These findings also suggest that we can sample flies for surveillance of existing and emerging pathogen and AMR threats in the environment.

Development of a rapid resistance assay to aid in mosquito control. Pyrethroids have been used in the control of mosquitoes for years. Due to their heavy use, many populations of mosquitoes became resistant to pyrethroids. Rapid detection of resistance allows for the proper pesticide selection for mosquito abatement. ARS researchers in Gainesville, FL, and collaborating scientists at the U.S. Department of Defense developed a novel, rapid assay for genetic assessment of pyrethroid resistance in the southern house mosquito, *Culex quinquefasciatus*. This assay is quick, inexpensive, and fits within existing assay systems for several species of mosquitoes. The assay is currently being tested in a Florida study and will soon be implemented in Louisiana.

Component 2: *Veterinary Entomology*

Attractant-impregnated sticky film for stable fly mass trapping. Stable flies feed on livestock and cause significant animal stress and loss of vigor. This leads to production losses, increased susceptibility to disease, and sometimes death. These flies are also a nuisance pest of domestic pets, zoo animals, and humans in some U.S. coastal areas. Current stable fly control methods are costly and ineffective . ARS scientists in Lincoln, NE, in conjunction with an industry partner, developed an attractant-impregnated adhesive tape for mass trapping stable flies. The traps can reduce stable fly induced stress and avoidance/defensive behaviors of confined cattle by up to 80 percent, and result in improved cattle weight gain and milk production. This technology contains no pesticides. Therefore, it is safer for the environment. It also has potential for reducing stable flies on pets, zoo animals, and on humans in coastal communities which currently do not have the ability to effectively control this pest using conventional pesticides or traps. An international patent was granted on this technology.

House flies carry and can potentially transmit bacterial pathogens associated with bovine respiratory disease. House flies are major nuisance pests at feedlots and are of concern to animal health since they acquire, harbor, and transmit numerous pathogens. Bovine respiratory disease (BRD) is an economically important and complex illness of cattle associated with several bacterial and viral species. It is not clear what role flies play in harboring and transmitting bacterial pathogens associated with BRD. ARS scientists in Manhattan, KS, collected house flies from a commercial feedlot where cattle were suffering from apparent respiratory illness. Two different methods were used to examine the prevalence of the three main BRD bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida,* and *Histophilus somni* in male and female flies. Using both methods, *M. haemolytica* was detected in 11.7 percent of house flies, followed by *P. multocida* (5 percent) and *H. somni* (3.3 percent). The presence of BRD bacterial pathogens in house flies suggests they can play a role as reservoirs and disseminators of the bacteria in the feedlot environment. Further, infected flies pose a risk transmitting BRD when they acquire pathogens from sick animals and subsequently associate with healthy animals.

Novel insecticide shows promise for fly pests of livestock. Heavy reliance on chemical control resulted in widespread resistance to almost all available marketed pesticides for house and horn flies. ARS researchers in Gainesville, FL, and Kerrville, TX, and researchers at Northern Illinois University, The Pennsylvania State University, and Cornell University evaluated the effectiveness of the novel insecticide fluralaner against house and horn flies resistant to two commonly used insecticides, imidacloprid and permethrin. When fed to flies in sugar bait, fluralaner was 23-fold more toxic to a susceptible house fly strain than imidacloprid and more than 117-fold more toxic to an imidacloprid-resistant house fly strain. It also out-performed permethrin when applied directly to the house and horn flies. At present, fluralaner is only registered for use on dogs and cats for flea control. These results indicate this material would be very useful against insecticide-resistant flies.

Toxicity of chitosan for house flies, horse flies, and blow flies. House flies, horse flies, and blow flies are important pests of humans and their associated animals. ARS scientists in Gainesville, FL, and researchers at the University of Massachusetts examined insecticidal properties of chitosan, a polysaccharide derived from chitin. Chitosan fed to adult house flies (*Musca domestica*), horse flies (*Tabanus nigrovittatus*), and blow flies (*Phormia regina*), was found to be toxic to all three. Chitosan appears to disrupt the microorganisms that live in the gut of the fly. It is easily made, commercially produced, non-toxic to mammals, biodegradable, and used in a range of agricultural applications. Chitosan shows promise as a new environmentally friendly pesticide against house flies, horse flies, and blow flies affecting humans and livestock.

Natural compounds as insecticides and fly repellents. Fly resistance to chemical pesticides is an increasing problem and highlights the need to develop alternatives to existing fly control chemical pesticides. ARS scientists in Kerrville, TX, demonstrated the effectiveness of a number of natural compounds, including essential oils and other botanical compounds, as repellents or insecticides in laboratory bioassays against various life stages of horn flies. Cinnamon oil, spearmint oil, citronellol, and limonene each exhibited repellent activity comparable or greater than N, N-Diethyl-meta-toluamide (DEET) to horn flies. Unlike DEET, these natural plant compounds also exhibited insecticidal activity against horn flies. These compounds can be incorporated into integrated pest management strategies to suppress horn fly populations associated with livestock production, reducing losses to livestock producers and potential annoyance to nearby human populations.

Virus infection makes flies lose their appetite. Previous studies have shown that *Musca domestica* salivary gland hypertrophy virus (MdSGHV) dramatically enlarges salivary glands and prevents or delays ovarian development in its adult host, the common housefly, and could potentially be used as a biocontrol method. The effect of the virus on the fly's natural food consumption, however, remained unexplored prior to this study, which was conducted by ARS scientists in Gainesville, FL, and researchers at the University of Massachusetts. Both virus-infected and control flies were provided a choice of an 8 percent sucrose solution or a 4 percent powdered milk solution to determine food preferences. Healthy females with developing ovaries continued to consume a sugar and protein diet while infected females fed predominantly on a sugar diet. Infected flies of both sexes consumed less food than healthy flies. Infected flies in the field may spend less time visiting food sources, which could reduce their survival and the risk of human pathogen movement.

Beauveria bassiana is only effective against house fly larvae during a brief time window. Using entomopathogenic fungi such as *Beauveria bassiana* to manage adult house fly populations shows promising results, but little is known about whether it can be used against house fly larvae. ARS researchers in Gainesville, FL, demonstrated that temperature and diet did not modify the effectiveness of *B. bassiana* treatments against fly larvae. Additional testing revealed fly larvae are only susceptible to the pathogen when they are very young, and very high fungal doses are required to kill them. The results show that *B. bassiana* can control adult flies effectively but that larval control with this biocontrol agent is prohibitively expensive.

New and quick method to identify fever ticks resistant to pyrethroids. Cattle fever ticks were eradicated from the southeastern United States but continue to re-infest parts of southern Texas. The presence of wildlife complicates eradication efforts by spreading ticks across the Mexico-United States border into Texas. Pyrethroids are a class of pesticide used to control tick infestations on cattle in Mexico and on U.S. wildlife. However, many tick populations in Mexico are resistant to pyrethroids. Quick and accurate diagnosis of pyrethroid resistance in the cattle fever tick is critical for selecting the appropriate pesticide to use. ARS scientists in Kerrville and Edinburg, TX, and Pullman, WA, worked with collaborators at Northern Arizona University and the University of Queretaro (Mexico) to develop a new and quick method to identify fever ticks resistant to pyrethroids. This assay can detect multiple changes in the gene coding for the protein targeted by pyrethroids and which can lead to resistance.

This molecular assay can be completed overnight while the traditional bioassay method takes 6 weeks to complete. The ability to quickly detect pyrethroid-resistant ticks allows decision makers to choose the proper pesticide to use on wildlife during Texas outbreaks.

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

Natural spread of a virus in imported fire ants. Introduced from South America, the red imported fire ant currently infests more than 128 million hectares of land in the United States and is estimated to cause damage exceeding $7 billion annually. *Solenopsis invicta* virus 3 (SINV-3) is an RNA virus specific for red imported fire ants that offers promise as a natural control agent. It infects queens and immature ants and results in a colony weight reduction of more than 50 percent. ARS scientists in Gainesville, FL, and researchers at Florida A&M University conducted surveys to determine the prevalence of SINV-3 in winged female fire ants to understand the possible natural spread of the virus through mating flights. Collections were made from five urban areas and five adjacent north Florida rural areas. SINV-3 was detected in winged females in nests from 7 of the 10 collection locations. The average infection rate of 44 percent was similar in rural and urban areas. Winged females were sampled because they mate aerially and disperse, founding colonies in new areas. Infected winged females may be the mechanism of SINV-3 spread throughout the fire ant community and may provide additional sustained control of fire ants in the United States.

A super colony of invasive ants in Florida. The tawny crazy ant is an invasive ant from South America that infests Florida and Texas and is spreading to states along the Gulf Coast. Extremely large populations of this ant inundate urban and natural landscapes, resulting in mass intrusions into buildings and reductions in ant biodiversity by displacing other native and invasive species. ARS researchers in Gainesville, FL, determined that tawny crazy ants did not fight with other tawny crazy ants from different nests located at the same site, or with ants from nests located as far as 270 miles away. In fact, small fragments of colonies from distant nests, including queens, congregated together in the same nests in laboratory tests. Tawny crazy ants are not territorial over large areas. These findings along with genetic data from earlier studies suggest that tawny crazy ants in Florida are part of a genetically closely related super colony across the southern United States and that the lack of territorial behavior facilitates resource sharing and the movement of worker ants and brood between colonies. These characteristics could be used in developing control strategies, including the spread of natural enemies such as pathogens and toxic baits being developed for their control.