Veterinary, Medical, and Urban Entomology (National Program 104)
Annual Report for FY 2018

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 translational, fundamental 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.

Thirty-nine permanent scientists in 11 projects conduct research in ARS laboratories located in 6 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;
Livestock Arthropod Pest Research Unit, Kerrville, Texas (3 projects).

Fiscal year funding for research conducted under the auspices of NP 104 approached $32 million, of which approximately $7 million of these funds were received through extramural agreements. The Deployed War-Fighter Protection (DWFP) Program continues to provide approximately $1.5 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.

The quality and impact of NP 104 research in 2018 was evidenced by the following research-related activities and products:

- 5 new invention disclosures and 4 patents filed;
- 5 patents issued;
  - Patent No. 9,795,114 Automatically detachable collar pendant system, issued October 24, 2017
  - Patent No. 9,796,975 Double-stranded ribonucleic acid as control against insects, issued October 24, 2017
  - Patent No. 9,832,973 Automatically-attaching collar clasps, issued December 5, 2017
  - Patent No. 9.950,994 Methods and compositions for pest control, issued April 24, 2018
  - Patent No. 10,017,538 Bioactive peptides having insecticide activity, issued July 10, 2018
- 1 new Cooperative Research and Development Agreement;
- 10 new Reimbursable and Trust Agreements;
- 16 new Interagency Agreements; and
- 18 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 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. NP104 scientists also assist U.S. Department of Defense (DoD) with research focused upon protection of warfighters and other personnel from arthropods that cause diseases in humans.

Scientists in NP 104 published 70 papers detailing their research findings in peer-reviewed journals such as American Journal of Tropical Medicine and Hygiene, Annals of the Entomological Society of America, Biocontrol Science and Technology, BMC Genomics, Environmental Entomology, Frontiers in Bioscience, Journal of the American Mosquito Control Association, Journal of Economic Entomology, Journal of Entomological Science, Journal of Insect Science, Journal of Medical Entomology, Journal of Wildlife Management, Journal of Vector Ecology, Medical and Veterinary Entomology, Nature Scientific Reports, Pest Management Science, PLoS ONE, Preventive Veterinary Medicine, Subtropical Agriculture and Environments, Ticks and Tick Borne Diseases, Transboundary and Emerging Diseases, and Tropical Biomedicine. 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, Columbia, Costa Rica, Ecuador, France, Greece, Israel, Italy, Kenya, Mexico, Morocco, New Zealand, Pakistan, Panama, South Korea, Sweden, Taiwan, Thailand, Ukraine, 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

The following scientists retired in 2018:

Dr. James Becnel, Mosquito and Fly Research Unit, Gainesville, FL.

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

The following scientists in NP 104 received prominent awards in 2018:

Dr. Adalberto Angel Pérez de León, Laboratory Director of the Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas, received the Outstanding Alumni Award from the College of Agriculture and Natural Resources, University of Wyoming, for being an advocate for issues affecting Wyoming.

Notable Research Accomplishments by Program Components

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

Widespread pyrethroid resistance in Florida mosquitoes that transmit Zika and Dengue viruses. Recent outbreaks of locally transmitted Zika and dengue viruses in Florida have placed more emphasis on the importance of integrated vector management plans for *Aedes aegypti*. ARS researchers at Gainesville, Florida, together with collaborators conducted a statewide examination of pyrethroid resistance in Florida *Ae. aegypti* populations and demonstrated that permethrin resistance and the genetic markers for resistance are widely present. Results showed all strains of *Ae. aegypti* were resistant although the strength of the resistance varied. This resistance information will be useful for improved mosquito control operations that need to select and apply the best pesticides to control these mosquitoes that transmit diseases to humans.

New control method for *Aedes aegypti* patented. ARS researchers at Gainesville, Florida, and collaborators have identified two dsRNA triggers that cause long-term reductions in reproduction of the Yellow Fever mosquito, *Aedes aegypti*. The effects of these triggers were expanded and confirmed with several methods. Both triggers target the ribosome which is

involved in most cellular systems. Targeting of these genes as a method for control of insects was patented as: Estep, A. S., Sanscrainte, N. D., & Becnel, J. J. Double-stranded ribonucleic acid as control against insects. Issued October 24, 2017.Patent # 9,796,975.

New strategies to control bed bugs. New strategies to control bed bugs are always in demand for both the public and companies involved in controlling these blood-sucking insect pests. ARS scientists in Beltsville, Maryland, in conjunction with university scientists in Towson, Maryland, showed that fumigation with a naturally-occurring chemical was useful in controlling both chemically-susceptible and chemically-resistant bed bug strains. This information will help industry personnel develop new bed bug control methods.

Prediction and early warning for elevated risk from Rift Valley fever in Africa. Rift Valley fever is a mosquito transmitted virus that causes serious and often fatal disease in cattle, sheep, goats, and humans in Africa and the Middle East. It has the potential for spread to animals, humans, and mosquitoes in the United States and other parts of the world. ARS researchers at Gainesville, Florida, forecasted and issued early warnings to the World Health Organization, Food and Agriculture Organization, and World Organization for Animal Health for elevated risk of Rift Valley fever outbreaks which subsequently occurred in four African countries (Sudan, South Africa, Kenya, and Rwanda) by analyzing global climate data with National Aeronautics and Space Administration and Department of Defense partners. The success of these forecasts resulted in increased surveillance and control efforts in affected countries, minimizing the impact of the disease in local populations of animals and humans and greatly reducing the risk that the disease would be spread to the United States and other locations in the world.

Novel carbamate pesticides for use against organophosphate resistant arthropods. Scientists at ARS in Kerrville, Texas, studied synthetic carbamates for inhibition of recombinant fly acetylcholinesterases (AChEs) containing known organophosphate resistance mutations in sand flies and mosquitoes. Novel synthetic carbamates are effective inhibitors at low concentrations, which means that they are candidates for control of organophosphate-resistant flies and mosquitoes, including organophosphate-resistant sand flies that spread leishmaniasis in the Middle East and mosquitoes that spread malaria in Africa.

Residual larvicide multiple environment pre-treatment for *Aedes aegypti*. The *Aedes aegypti* mosquito is a key vector of prominent viruses including dengue, chikungunya, and Zika and is very difficult to control. ARS researchers at Gainesville, Florida, and United States and international collaborators demonstrated the efficacy of four common larvicides applied as a residual to dry containers that would later be flooded with water. Studies were conducted across a range of environments relevant to the global spread of this mosquito. Results indicate that pre-treatment of dry habitat vulnerable to flooding can extend the impact of mosquito control agencies that otherwise must treat flooded areas cyclically or in response to mosquito populations that are already developing. These studies were published as a series of papers in the Journal of the American Mosquito Control Association.

Superior tick repellency of ant-derived compounds. Lyme disease and other tick-transmitted diseases are the most important vector-borne diseases affecting Americans in the northeastern United States. ARS scientists in Beltsville, Maryland, and Stoneville, Mississippi, discovered superior repellency of several ant-derived compounds against lone star ticks that cause disease in humans, livestock, and other animals. This information will help federal, university and pharmaceutical scientists to develop new natural repellent formulations for personal protection, thereby reducing the risk of tick bites and therefore, disease transmission.

Natural repellents for protection from the bites of sand flies. There are essential oils that are conditionally exempt from EPA registration because they are considered minimum risk pesticide products under 40 CFR 152.25. Their major components were tested at ARS in Kerrville, Texas, and some were found to be strong repellents of the Old World sand fly, *Phlebotomus papatasi*. This biting fly is a primary vector of cutaneous leishmaniasis, a disease impacted and significantly reduced readiness of deployed military personnel in Iraq and Afghanistan. Repellency was recorded at concentrations substantially lower than the standard contact repellent, *N,N*-diethyl-*meta*-toluamide (DEET). The conditionally exempt status from EPA registration allows immediate use of these oils as area, spatial, or personal repellents.

Spatial repellent treated military materials for use against mosquitoes and sand flies in multiple environments. Military materials commonly used in the field are important substrates for residual pesticides that may protect troops and other personnel from nuisance and disease-vector biting insects. ARS researchers at Gainesville, Florida, and United States and international collaborators demonstrated that two types of camouflage netting and one type of material used to retain fill in blast-protection walls can be effectively treated with the spatial repellent/toxicant transfluthrin. Strips of these materials treated with transfluthrin greatly reduce or eliminate populations of both mosquitoes and sand flies in small 1—3 m diameter protected spaces across a range of militarily-relevant environments including tropical, desert, temperate, and Mediterranean. Importantly, strips are easily transported and attached to existing camouflage net or blast-protection structures by minimally trained personnel, creating a rapid shelter from biting insects without having to wait for intervention by mosquito and vector control units.

Mosquito control using sterilized male *Aedes aegypti* mosquitoes in Florida. The *Aedes aegypti* mosquito is a key vector of prominent viruses including dengue, chikungunya, and Zika and is very difficult to control. ARS researchers at Gainesville, Florida, and university and mosquito control district collaborators developed a method to sterilize male *Ae. aegypti* mosquitoes and release them in large numbers to overwhelm wild populations of fertile males. The success of this method depends on sterilized males not only surviving in the wild, but also exhibiting mating behavior competitive with local, wild males such that wild females mate more often with sterilized males. Mark-release-recapture trials in the field with sterilized males produced satisfactory recapture rates, indicating survival in the wild, and led to direct observations of marked males in mating swarms potentially attractive to local wild female *Ae. aegypti* and the opportunity for subsequent population suppression.

Natural product tick repellents and attractants. Alternatives to the common use of, or technologies that can be used together with synthetic chemicals to kill ticks are needed to improve integrated approaches for tick control. The use of repellents and deterrents is a useful

tactic to prevent tick bites and mitigate the risk of tick-borne disease transmission. Laboratory tests by ARS scientists in Kerrville, Texas, showed that *para*-anisaldehyde deterred the movement of the immature stage of the lone star tick. One outcome of these research findings is that *para*-anisaldehyde may be developed to deter tick movement up grass stems thereby interfering with their ability to find a host, or if applied to animals deter the ticks from biting and blood feeding.

Natural compounds as insecticides for biting fly control. *Para*-anisaldehyde is a natural compound used commercially as a flavoring ingredient and is found in American cranberry, anise oil, fennel and vanilla. ARS researchers at Kerrville, Texas, found that *para*-anisaldehyde exhibited significant insecticidal activity against several fly species, including the horn fly, stable fly, housefly and at least one species of sand fly, *Phlebotomus papatasi*. The compound also deterred female flies from laying eggs activity when this compound was incorporated into fly larval medium, suggesting possible use as an ovipositional deterrent for treatment of round hay bale cattle feeding stations, which are believed to be a prime reproductive environment for stable flies.

Ants as predators of the lone star tick in Texas. Ants have been reported as being effective predators of hard ticks. Field experiments conducted by ARS scientists in Kerrville, Texas, in west, central, and south Texas, including the south Texas coastal plains, used lone star tick eggs, larvae, nymphs, unfed adults, and replete adults to determine whether or not ants under natural conditions fed on them. While other baits such as small pieces of meat and dead house flies attracted foraging ants, none of the species of predatory ants encountered were attracted to any lone star tick life stage. In some instances, harvester ants pulled replete adult ticks away from their colonies and carried tick eggs away to dispose of them beyond the circular colony clearing. The likely reason for the lack of predation against lone star ticks was determined to be chemically-based. This research shows that more field studies are required to ascertain the role of ants as predators across tick species in Texas.

Component 2: *Veterinary Entomology*

Sequencing of the genome of the longhorned tick. The longhorned tick, *Haemaphysalis longicornis*, is a known serious pest of livestock in Asia. It is an aggressive biter with a diverse host range, can reproduce asexually, and is a vector of several debilitating agents of livestock and human diseases. This tick has recently established populations in at least nine U.S. states. As part of an emergency response to assist USDA-APHIS, ARS scientists at Kerrville, Texas, collaborated with researchers at Texas A&M University AgriLife in College Station, Texas, to sequence the genome of the tick. The completed genome opens new avenues of research on tick control methodology, including vaccine development and detection of pesticide resistance-associated genes.

Epidemiological modeling to improve integrated pest management of mosquitoes. ARS scientists in Manhattan, Kansas, in collaboration with Kansas State University researchers quantified the risk to the United States of Japanese encephalitis virus, a disease that is currently not in the United States. The introduction risk is currently minimal from airplanes and cargo ships based on a risk assessment analysis. Factors that are input into the analysis are the current

trade and transportation routes and environmental conditions; analysis of the mosquitoes and their hosts; and analysis of the mosquito infection, dissemination, and transmission rates. The findings from this study indicate that the mosquitoes responsible for disease transmission play a more significant role than originally realized. The new model results highlight how the spread of disease relies more strongly on the mosquitoes and their behaviors. The results are useful to epidemiologists, state and federal mosquito management districts, and health care specialists to improve surveillance and population management of disease vectors to prevent disease outbreaks in humans and livestock.

Nilgai spread of cattle fever ticks in south Texas. Globally, exotic and invasive wildlife species that are hosts for ticks impede efforts to protect local livestock from ticks and tick-borne diseases. Nilgai movement across the Gulf Coast in south Texas complicates efforts to keep the United States cattle herd free of cattle fever ticks. Nilgai are exotic bovine relatives, introduced for hunting in Texas. They are hosts for tick and tick-borne diseases. Cattle fever ticks transmit bovine babesiosis, a disease which is deadly to cattle. ARS scientists in Kerrville, Texas, were part of a collaborative effort to update the United States Geographic Information System (GIS) database on historical tick infestation data, host source data, and geographical data related to the increasing populations of nilgai in south Texas. Studies to enhance the understanding of how nilgai spread cattle fever ticks through the landscape in south Texas revealed that: 1) they are unlikely to cross 1.25 m high cattle fences located parallel to paved highways; and, 2) females are more likely to disperse the ticks than males. This information has been integrated into the database to compare activity patterns between nilgai and white-tailed deer, which is a native wildlife species and cattle fever tick host that also compromises the cattle fever tick-free status of the United States. The outcomes of this research can be used to enhance integrated strategies for the management of cattle fever ticks infesting nilgai and white-tailed deer.

Network modeling of emerging disease threats to the United States. ARS scientists in Manhattan, Kansas, in collaboration with Kansas State University researchers developed a model to assess the hypothetical spread and impact of Japanese encephalitis virus (JE) and Rift Valley fever virus (RVF) after an introduction to the United States. In the event of an outbreak of JE or RVF in the United States, understanding the roles of the hosts and vectors in geographic spread will be essential. Network modeling was used to model the spread of viruses between farms and states in the Midwest, Texas, and the eastern seaboard. The model was developed using data from outbreaks in South Africa and then adapted to farms in the United States. The data for input were mosquito species (vectorial capacity), weather (temperature and humidity), and host species (humans and other animals). Geographic locations and trade between farms were also considered. In the case of Japanese Encephalitis, migratory birds were also considered since they are reservoirs for long-distance migration. This information is important for states and federal emergency planners who may need to rapidly react to introductions of exotic pathogens to protect the food supply.

Publication and release of the assembled horn fly genome sequence. The genome of the horn fly contains the biological blueprint necessary to allow the fly to develop and survive over its lifetime, including how the fly responds to external stimuli and threats such as pesticides. ARS scientists at Kerrville, Texas, collaborated with researchers at National Center for Genome Resources and Texas A&M University to sequence, assemble, and annotate this fly's genome.

Genes involved in pesticide resistance and metabolism, sex determination, and blood feeding were identified and described. This new comprehensive dataset is being used to identify the various mechanisms used by the fly to resist pesticides and develop novel fly control technology that will be relevant to agriculture.

Methods to treat cattle fever tick infestations in nilgai. Nilgai antelope, native to India and established in the rangelands of south Texas and northeast Mexico, are highly mobile hosts of cattle fever ticks and implicated in the widespread movement of this serious livestock pest that is also a vector of the microbes causing bovine babesiosis, and anaplasmosis. There are no methods to kill cattle fever ticks infesting nilgai. A remotely activated sprayer developed through collaborative efforts by ARS scientists in Edinburg and Kerrville, Texas, to treat cattle fever tick infestations in nilgai is activated when nilgai cross under established fence crossings or visit common latrines. There is a commercially available roundworm that is able to cause death of insects. This nematode, delivered in a spray-based apparatus, has been evaluated as a technology to kill cattle fever ticks. The nematode is native to South Texas and non-toxic to mammals and other vertebrates. Large-scale, *i.e.* ~40,000 acres, deployment and testing of the nilgai sprayer on ranchland in Willacy Co. is underway in cooperation with Texas A&M Kingsville, Caesar Kleberg Wildlife Research Institute.

Improved control for adult house flies. One method of house fly control is the use of the fungal pathogen *Beauveria bassiana*. ARS researchers at Gainesville, Florida, and researchers at University of Florida compared adult fly susceptibility to single and combined treatments of *B. bassiana* and the bacteria *Photorhabdus temperata*, *Serratia marcescens*, and *Pseudomonas protegens*. Bacteria killed flies faster than *B. bassiana* when injected. *B. bassiana* and *P. protegens* caused mortality when applied topically. An exotoxin may cause *P. protegens* mortality. *P. protegens* killed faster than *B. bassiana* but with a lower mortality rate. Results suggest that the two pathogens used together provide better fly control than either of them alone.

Mathematical modeling to better understand geographic expansion of disease outbreaks. ARS scientists in Manhattan, Kansas, in collaboration with Kansas State University researchers examined past human case data as part of a retrospective analysis of the spread of disease outbreaks. Human case data are valuable inputs for mathematical and geographical modeling to better understand the mechanisms that drive the geographic spread of disease during disease outbreaks. In this study, past cases of Lyme disease were used to model the spread of Lyme disease in Connecticut. The analysis indicated that there were two introductions of Lyme disease pathogens into Connecticut and the spread occurred at a linear rate. Pathogen introduction to new areas resulted in peak numbers of yearly cases which declined after establishment to stable yearly transmission. This same type of model was then applied to mosquitoes and the potential spread of Japanese Encephalitis. This was useful because it allowed for predictions of future cases based on environmental parameters.

Autodissemination of pyriproxifen for house fly control. The insect-growth regulator, pyriproxyfen prevents fly development past the pupal stage. ARS researchers at Gainesville, Florida, and researchers at University of Haifa, used flies to apply this insect-growth regulator to their egg-laying sites by exposing active-coated flies at different proportions in laboratory tests with animal manures. A control level of 90% in most U.S. manures was achieved from just

10-20% of pyriproxifen-coated flies. In Israel, mortality was not as successful in cow manure but very successful in poultry manure where just 10% pyriproxifen-coated flies produced high mortality. Results confirm that autodissemination of this insect growth regulator using the active coating concept may be practical depending on manure type and target population size.

Reduction in *Musca domestica* reproduction by dsRNA-mediated gene knockdown. The house fly (*Musca domestica*) is a major pest of humans and animals throughout the world. ARS researchers at Gainesville, Florida, and collaborators have identified a gene that when silenced with a dsRNA trigger results in long-term reductions in reproduction of the house fly. This study demonstrates that exogenous dsRNA is specifically effective and has potential efficacy as a control intervention in adult house flies. Further work is required to develop effective methods for delivery of dsRNA to adult flies.

Impact of integrated carrizo cane management on cattle fever ticks in the Eastern sector of the United States-Mexico transboundary region. Carrizo cane, an invasive weed, creates an environment conducive for cattle fever ticks and impairs U.S. Border Patrol operations in some sectors of the Rio Grande that also form part of the Permanent Quarantine Zone (PQZ) managed by the Cattle Fever Tick Eradication Program (CFTEP). Methods for integration of mechanical topping technology with biological control agents were transferred by ARS scientists in Edinburg, Texas, to the U.S. Border Patrol, USDA-APHIS-Veterinary Services, and Kunafin Insectary for management of carrizo cane along 350 river miles in the CFTEP PQZ along the Rio Grande. Topping of cane at 3 feet with specialized tractors causes growth of cane side shoots, which are readily attacked by the carrizo cane wasp and scale biocontrol agents. This method provided immediate visibility of the international border for law enforcement agencies and causes carrizo cane long-term suppression, which leads to re-emergence of desirable native vegetation. Establishment of native vegetation along the Rio Grande increases population levels of native cattle fever tick predator ants and beetles. This integrated solution meets the needs of Federal agencies tasked with managing this invasive weed along the international border.

Attractant-impregnated sticky film for stable fly mass trapping. Biting flies harm local livestock, companion animals, and humans through bites that lead to infections or inflammation. ARS scientists in Lincoln, Nebraska, identified attractant compounds associated with the ability of flies to locate cattle in the environment. When incorporated into traps, these compounds increase the capture of biting flies. This technology has been developed into a novel attractant-impregnated sticky film. A prototype product was successful when used for stable fly mass trapping in cattle feedlots. Traps that efficiently lure and collect flies lead to improved population surveillance and can reduce biting fly damage to livestock in situations where few other options are available.

Understanding southern cattle fever tick survival in the environment. The southern cattle fever tick (SCFT) is an economically destructive disease vector that transmits the microbes causing bovine babesiosis, a deadly disease of cattle. These ticks can spend 80–90% of their life cycle as a seed tick (larval stage) after emerging from eggs laid by the mother in the environment where they wait to latch on cattle, or another host to complete the parasitic phase of their life cycle. A two-year environmental study conducted in a south Texas pasture by ARS scientists in Edinburg, Texas, showed that survival rates varied among seasons with the overall highest

populations recorded in the spring and the lowest in the fall. Larger numbers of ticks were collected from exposed habitats in the winter whereas canopied habitats in the summer had ten-fold larger seed tick numbers. In the spring, exposed and canopied habitats showed no difference in tick larval survival rates. The interaction between season and habitat strongly influence the survival of seed ticks waiting to latch on a host with relative humidity being a key weather variable. This information can be used to develop effective interventions that target seed ticks when they are off the host trying to survive in the environment.

Natural products for tick control. Cattle fever and other hard ticks can develop insecticide resistance to synthetic acaricides. Natural products were tested by ARS scientists in Kerrville and Edinburg, Texas, to determine if they can be used to kill ticks, especially those that are resistant to commercially available synthetic acaricides. The compounds *para*-anisaldehyde, a botanically-based compound produced by fennel, cranberries, vanilla, and other plants killed and impaired reproduction capability of the lone star tick. The commercial dust products Surround®, containing kaolin, and CimeXa™, containing silica, which cause desiccation of pests were bioassayed for activity against the lone star tick. CimeXa™ was relatively more effective than Surround® against the lone star tick in laboratory tests. When tested in the field, CimeXa™ attained >95% control within 24 hours against natural populations of the immature stages of the Gulf Coast tick when applied to cordgrass during relatively high wind conditions. This finding indicates that this inert and organic product with potentially long-residual activity has properties that distinguish it from other pesticidal products used for hard tick control.

Non-nutritive house fly bait using artificial sweeteners. The entomopathogenic fungus, *Beauveria bassiana*, is an effective house fly bait when mixed with sugar. Because this bait takes several days to kill flies, and sugar tends to enable flies to live longer, this is not the best combination for a house fly bait. ARS researchers at Gainesville, Florida, and researchers at Northern Illinois University altered the bait by replacing sugar with the artificial sweeteners, xylitol and erythritol. The sweeteners, used in human foods, have little nutritional value. Flies fed avidly on the sweeteners which effectively delivered *B. bassiana* to kill flies. Thus, the bait did not provide flies with nutrients that promote increased fly life. These results lead to improved house fly control when using *B. bassiana*.

Increased efficiency and reduced cost of screwworm production. Improved rearing procedures were developed by ARS scientists in Kerrville, Texas, and are being incorporated at the production facility for large-scale rearing of screwworm flies for sterile male release. Some of these procedures lead to decreased release of toxic ammonia gas within the production plant. Another approach that has been developed is production of a transgenic male-only screwworm strain. This strain is undergoing evaluation in a small field-scale release to test its capacity to successfully compete for mating with wild screwworm flies. If fully successful, replacement of rearing fertile screwworm lines with the transgenic male-only strain is expected to reduce fly production costs by up to 50% and potentially eliminate requirement for irradiation to sterilize the flies, further improving fly vigor and competitiveness in the sterile male release program.

Ecology of hard ticks in the south Texas coastal plains. The ecology of the recent incursion of the southern cattle fever tick into parts of the south Texas coastal plains, which include a wildlife corridor, remains to be fully understood. Field studies by ARS scientists in Kerrville, Texas,

revealed interactions between soil conditions involving salinity and moisture that strongly affect hard tick populations in the wildlife corridor caused by saline tidal and storm surge action, humidity, and the predominance of the mud flat fiddler crab. These crabs are efficient tick egg predators and saline water destroys tick eggs such that hard tick populations on highly saline soils are negligible whereas greater tick populations were observed in areas with low-salinity soil. Ticks were scarce in areas where the sea ox-eye plant typically grows in dense near-monocultures on highly saline soil that are identifiable by the plant's distinctive color, which enabled the production of a Geographic Information System map to show the distribution of those areas relative to the wildlife corridor. Examination of mammalian tracks and dung in the corridor helped determine wild host animal activity and revealed that some animals disperse ticks in high salinity and low salinity areas where tick survival apparently depends on saline water and crab predation levels on tick eggs. Most ant species appeared unable to detect lone star tick eggs, which could explain why tick populations in the presence of ants, including the red imported fire ant, can be high relative to saline soil areas subject to tides, storm surges, and crab predation. Identifying areas where hard ticks, particularly one-host ticks such as cattle fever ticks, are sparse can help streamline surveillance activities conducted by the Cattle Fever Tick Eradication Program and other stakeholders pursuing tick control in the south Texas coastal plains.

Parasitoid-based control of carrizo cane. Carrizo cane is an exotic plant that is problematic because of its effect on water flow of rivers, streams, and canals displacing indigenous riparian plant communities. It is extremely difficult to control with herbicides, fire, and mowing due to its tendency to grow back quickly after treatment. The carrizo cane wasp, *Tetramesa romana*, was introduced from the Mediterranean region and is used in south Texas for integrated carrizo cane biocontrol. Studies in the Texas Hill Country by ARS scientists in Kerrville, Texas, showed that the wasp does not attack carrizo cane stalks, even when young, and while lateral shoots growing from the cane nodes are used for oviposition, the larvae developing within do not necessarily kill the shoots. Under these conditions, shoots might grow longer when infested and proliferate, and wasps did not reduce stalk density and growth infesting the carrizo cane for only a short time during the lifetime of the plant. This study highlighted the need for research to understand how different ecosystems can influence the performance of a parasitoid on its plant host for biocontrol purposes, and that integrated biocontrol strategies are a more effective approach to manage exotic invasive weeds like carrizo cane.

The Knight Stick fly trap remains efficient when surrounded by hot grass. Knight Stick (KS) sticky fly traps surrounded by protective squares of electric fence placed close to animal hosts capture up to ten times more stable flies. ARS researchers at Gainesville, Florida, and researchers at Smithsonian's National Zoological Park, Washington, District of Columbia, evaluated a range of types of enclosure materials, including hot grass, which conducts electric current and is practically invisible from a distance. Hot grass performed well and could be used in place of standard fencing wire with no reduction in effectiveness. This technology can be used at zoos, and by beef and dairy cattle and equine industries.

Sequencing of the genome of the biting midge (*Culicoides sonorensis*). Female *Culicoides sonorensis* biting midges harbor and transmit viral pathogens that cause sickness and death to ruminant livestock and deer. ARS scientists in Manhattan, Kansas, worked with a diverse group

of national and international researchers to sequence and annotate the genome of this important vector. New genetic resources such as this genome will significantly advance research capabilities by providing access to genetic targets to develop novel pesticides and repellents. Further, identification of genes involved in key defense processes will help elucidate the molecular basis of vector competence, i.e. the midge's ability to transmit pathogens.

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

Red imported fire ant detection device. Fire ant introductions at United States ports and at quarantine boundaries are a constant problem. Ants present in cargo must be rapidly identified to minimize shipping delays. In response to this need, a rapid, field-portable kit capable of identifying fire ants by untrained personnel was developed by ARS researchers at Gainesville, Florida, and APHIS researchers at Biloxi, Mississippi. The kit is based on the lateral flow immunoassay technology familiar in home pregnancy tests. The test provides an answer within ten minutes without any prior training or knowledge. Agdia, Inc. (https://orders.agdia.com/invictdetect-isk-49700-0010) has acquired the Biological Material License for the monoclonal antibodies in the test from the USDA and has begun commercially manufacturing the kits under the trade name, INVICTDETECT.

New sample preparation method for improved detection of volatile compounds in insects. In the past two decades, one technique, headspace solid-phase microextraction has been increasingly popular for insect pheromone analysis. The overall amount of volatiles that can be collected for chemical analysis affects the concentration of volatiles in head space. Essentially, this is largely determined by amount of volatiles insects indeed release into the head space. In many cases, active insect volatiles, such as insect pheromones, are produced in very low quantities. Any practices that can facilitate the release of volatiles into the headspace will facilitate the detection and identification of the volatiles. An innovative sample preparation method for Headspace Solid-Phase Microextraction (HS-SPME) was developed by a scientist in ARS, Stoneville, Mississippi, which significantly improves the detection and analysis of insect volatiles. In this method, a freeze-thaw process was applied to insect samples before the conventional solid-phase microextraction. The application of this innovation may significantly facilitate the identification of insect semiochemicals, such as insect pheromones. Freeze-thaw sample preparation method improves detection of volatile compounds in insects using headspace Solid-Phase Microextraction.

Environmentally benign benzoates as effective toxins against red imported fire ants. Due to their aggressiveness and venomous sting, red imported fire ants are a significant threat to humans, wildlife, crops, and livestock. Like many other insect pests, the management of red imported fire ants heavily depends on the application of synthetic insecticides. There is an increasing desire to use toxicologically and environmentally benign chemicals in insect pest management. ARS scientists in Stoneville, Mississippi, evaluated a list of benzoate compounds using topical and fumigation toxicity bioassays and demonstrated the potent contact toxicity of benzylbenzoate, hexylbenzoate and pentylbenzoate; and fumigation toxicity of methyl-3-methoxybenzoate, methyl-3-methylbenzoate, methylbenzoate, vinylbenzoate, and ethylbenzoate against red imported fire ants. With the exception of methyl-3-methylbenzoate, methyl-3-methoxybenzoate, and vinylbenzoate, all of these benzoates occur in nature.

Hexylbenzoate has neither known Occupational Safety and Health Administration (OSHA) hazards nor aquatic toxicity, and methyl 3-methoxybenzoate is not considered a hazardous substance, indicating a great potential for their application in fire ant management. This research has led to a Provisional Patent Application which was filed in 2018.

New fire ant species invades Hawaii. A single ant was taken from a flower basket before the basket was put in a freezer to kill nestmate ants. The ant was tentatively identified in Hawaii as the red imported fire ant, *Solenopsis invicta*. Since this ant species is one of the worst world-wide invasive pest ants and it has not been established in Hawaii, it was important to obtain positive confirmation. The single ant specimen was sent to ARS researchers at Gainesville, Florida, where a combination of biochemical and molecular techniques was applied to identify the ant as *Solenopsis xyloni*, a native fire ant in the continental United States, and not the red imported fire ant, *S. invicta*. The molecular and biochemical tools that allowed this determination were made possible through decades of ARS research to develop novel identification methods for species in this ant group that is very difficult to separate using classical taxonomy. Global trade is spreading the red imported fire ant to other countries in the Asia-Pacific area where these tools will continue to be in demand.