Action Plan
National Program 104 – Veterinary, Medical, and Urban Entomology
(August 2019 – August 2024)

Vision Statement: The vision for the program is a world where humans and other animals are safe from harm inflicted by arthropods.

Mission Statement: The mission of National Program (NP) 104, Veterinary, Medical, and Urban Entomology, is to eliminate arthropod vectors and the diseases that they transmit to livestock, humans, and other animals and to nullify their economic impact.

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.

Relationship of This National Program to the USDA Strategic Plan:

This Action Plan outlines research that supports Objective 2.3 in the USDA Strategic Plan for FY 2018-2022. Strategic Goal 2 is to maximize the ability of American agricultural producers to prosper by feeding and clothing the world. Within this Strategic Goal, Objective 2.3 is to protect agricultural health by preventing and mitigating the spread of agricultural pests and disease. Controlling the pests and diseases of humans, livestock, and structures is the major thrust of the NP 104 Action Plan.

Relationship of this National Program to the USDA Research, Education, and Economics (REE) Action Plan:

This NP 104 Action Plan outlines research that supports the 2014 REE Action Plan Goal 1: Sustainable Intensification of Agricultural Production, Subgoal 1B: Crop and Animal Health and Goal 2: Responding to Climate and Energy Needs, Subgoal 2A: Responding to Climate Variability.

Relationship of This National Program to the USDA Agricultural Research Service (ARS) Strategic Plan:

This NP 104 Action Plan addresses the high level goals and objectives of the 2012-2017 ARS Strategic Plan, specifically, ARS Strategic Goal Area 4: Animal Production and Protection, and Strategic Goal Area 4.2: Prevent and Control Pests and Animal Diseases that Pose a Threat to Agriculture, Public Health, and the Well-being of American Citizens. There is a performance measure that defines the targets for NP 104 research within the USDA-ARS Strategic Plan:

> **Performance Measure 4.4.2:** Provide scientific information to protect animals, humans, and property from the negative effects of pests and infectious diseases. Develop and transfer tools to the agricultural community, commercial partners, and government

agencies to control or eradicate domestic and exotic diseases and pests that affect animal and human health.

Introduction

Damage and disease associated with biting and stinging arthropods affect humans and livestock in the United States and around the world. Economic losses from arthropod damage, including crop losses, exceed one hundred billion dollars annually. Human globalization, international trade, local movement, and altered ecosystems facilitate the introduction of new disease vectors and pathogens into the United States, promote new parasitic arthropod-wildlife-livestock interactions, enable atypical arthropod vector-host-pathogen interactions, and expose humans to new vectors and pathogens. The research supporting Performance Measure 4.4.2 seeks to reduce arthropod damage to animals, humans, and structures. This work will target (1) arthropods transmitting pathogens that cause diseases in humans and animals, (2) pests that directly harm human health by feeding or stinging, and (3) nesting pests that damage structures. Due to the interconnection between the health of animals, people, and the environment, the program employs a "One Health" approach, which is the collaborative effort of the human health, animal health and environmental health communities. Through this collaboration, USDA supports sustained health outcomes for both animals and people.

Big Data. NP 104 conducts research that requires big data capabilities. This research includes:

- Forecast models for disease outbreaks based on environmental or climatic events;
- Databases and apps used to manage cattle fever outbreaks;
- reverse vaccinology, an approach that employs bioinformatics to screen the entire pathogenic genome. This approach led to development of a vaccine for Serogroup B meningococcus in 2000;
- Genomic studies, *e.g.* genome sequencing of arthropods of medical and veterinary importance that helps identify and elucidate gene activities in target organisms. Genomic techniques provide information on population genetics and support the following:
    - identifying invasive insect source populations and migration pathways;
    - the development of gene targets for disruption or RNAi gene suppression;
    - identifying pest species that are difficult to identify morphologically; and
    - more rapid discovery of natural enemies using next generation sequencing and metagenomics.
- Research on fly, tick, and ant metagenomes (the entire genomic complement found in the environment) and microbiomes (the universe of microbes living in association with each pest). These studies generate information about pathogens and commensal and symbiotic microorganisms that can be used to solve agricultural problems associated with flies, ticks, and ants. Scientists have worked with U.S. university partners and international institutions to generate ‘omic resources such as transcriptomes, microbiomes, and genomes for a number of livestock and human pests, including biting midges, mosquitoes, and house flies. Mosquito mitochondrial genomes sequenced using nanopore technology support the development of databases for rapid identification of field samples. Sequencing of pooled

field mosquito samples is performed for the real-time identification of hosts and pathogens. RNA-Seq is valuable for gene expression analysis of pathogens that cause disease in humans, livestock, and other animals.

Artificial Intelligence. NP 104 conducts research that involves artificial intelligence (AI). Robots and drones used for cattle fever tick surveillance and monitoring are an offshoot of new management and production strategies rooted in precision agriculture. Advanced neural networks (ANN) led to the discovery of new long-lasting mosquito repellents and more potent and selective insecticides. AI facilitates translating digital animal monitor outputs into behaviors associated with stress and defense and is also used to interpret digital imagery for quantifying ectoparasite infestation levels. Researchers use systems networks and mathematical modeling to model the spread of pathogens in U.S. vector-borne disease introduction scenarios. They also use particle filters and learning algorithms to combine disease data and environmental parameters to retrospectively determine basic reproductive rates and transmission parameters for mosquito vectors that infect humans with pathogens that cause disease.

Globalization: Globalization is leading to a consolidated world ecosystem characterized by the increased movement of people and products. Security challenges resulting from a globalized economy include preventing the introduction of exotic pest species and pathogens that damage humans, plants, animals, and infrastructure. In recent years, new mosquito-borne pathogens, *i.e.*, Chikungunya and Zika viruses, were introduced into the United States. The New World Screwworm, a pest completely eradicated from the United States by 1985, was rediscovered in the Florida Keys in 2016; by April 2017, ARS and USDA-APHIS had successfully eradicated this pest from the United States once again. In 2017, the invasive East Asian tick (*Haemaphysalis longicornis)* was discovered in New Jersey and has since been found in eight other states; studying archived samples, scientists determined the tick entered the United States before August 31, 2010. The invasive red imported fire ant was introduced into the United States in the 1930s, but the rate at which its U.S. range was expanding declined over the past few decades. However, within the last 12 years it has been detected in Australia, Taiwan, and mainland China and several port introductions were recently found in Japan and South Korea. These are just a few examples of how globalization facilitates the spread of arthropods and the diseases they transmit. These introductions and reintroductions reinforce the need for new and improved strategies and tools to detect, survey, control, and monitor arthropod pests to protect against diseases and other harmful damage.

*Altered Ecosystems and the Impact on Vector-Borne Disease***:** Ecosystem components include physical habitats, potential hosts, and pests; the characteristics of physical environments significantly affect the potential establishment and success of an invasive arthropod species or pathogen. The pathogenic landscape concept describes and explains ecosystem attributes affecting spatial variations in arthropod-borne disease risk or incidence; for instance, invasive weeds can facilitate the survival or invasion of exotic arthropod disease vectors. In some cases, small changes in climate dramatically affect the distribution and range of a pest species, which in turn affects a vector's ability to sustain disease cycles. Many distributions and ranges for established pests and vectors are based on climatic conditions, while urbanization and other human activities alter pest ecosystems, which affect the ability of pest populations to colonize and persist in specific locations and ecosystems.

NP 104 conducts research with cooperators to develop models that predict disease risks based on vector populations and environmental conditions. Maintaining disease cycles requires certain conditions that may help pest vectors and hosts reach threshold populations supporting sufficient vector access to hosts. For instance, though Zika and Chikungunya viruses were recently introduced into the United States, several factors probably reduced the local transmission of the pathogens that cause these diseases. These factors included insufficient populations of *Aedes aegypti* mosquitoes, which spread diseases, and insufficient contact between these vectors and their human hosts. The latter results from a combination of circumstances, including screened windows, availability of air conditioning inside dwellings, and use of mosquito repellents to prevent mosquito bites while outdoors.

The physical environment can affect other NP 104 species of concern in similar ways. Cattle Fever ticks on both sides of the Rio Grande transmit babesiosis (cattle fever) to livestock, but efforts to eradicate the tick is complicated by interactions with its feral hosts, white-tailed deer and exotic nilgai (Asian antelope). There are no standard treatment methods to control ticks on nilgai, which are highly mobile and contribute to the dispersal of cattle fever ticks beyond the Rio Grande in south Texas. Control efforts are further impeded by stands of invasive Spanish cane known as Giant Reed (*Arundo donax)* that provide shelter for cattle fever ticks. Another example of the environment impacting arthropod dispersal is through changes in average temperature. In recent years, a small increase in the average temperature along Costa Rica mountain ranges and changes in economic practices have resulted in an explosion of stable flies at pineapple and coffee plantations and on livestock at nearby ranches and dairies. U.S. producers have also reported an increase in stable fly populations in recent years. Climate change has a powerful influence on the distribution of invasive pest ant species. For example, the Asian needle ant was first reported in the United States in 1930, but only in the past 8 years have their populations exploded, causing them to be classified as an invasive pest ant. Suitable habitat for this ant is expected to increase by 75 percent around the world in the next 50 years due to climate change.

Cross-Cutting Research. The Veterinary, Medical, and Urban Entomology National Program (NP 104) conducts cross-cutting research with other ARS national programs. Greater livestock protection is achieved by research that results in disease reduction (a focus of NP 103: Animal Health) in concert with NP 104 research on arthropod control for reducing direct damage and stress to animals. A more specific example of the association between NP 104 and NP 103 is in their aligned focus on livestock disease vectors, (biting midges, mosquitoes, ticks) and the diseases that they transmit, *e.g.* Blue Tongue, Rift Valley fever, and babesiosis. Scientists in NP 104 cooperate with scientists in the European Biological Laboratory (EBCL) on Crop Protection and Quarantine (NP 304) to develop solutions for protecting deployed U.S. military personnel. Another example of cross-cutting research is the development of entomopathogenic fungi for mosquito control; this NP 104 work is linked to a Product Quality and New Uses (NP 306) project focused on new microbial and plant-based agents for mosquito control. All these efforts lead to solutions that increase and enhance animal production (a focus of NP 101: Food Animal Production).

Stakeholder Input. A stakeholder workshop webinar/conference call in February 2018 gave NP 104 stakeholders from a wide range of sectors an opportunity to provide input on priority research foci for the program. Their input was carefully considered, and a significant effort has been made to address as many of their concerns as possible in this plan. One of the more difficult decisions made concerning prospective research projects was to remove bed bug research from the current plan and redirect NP 104 resources to tick research, including ticks of medical importance and invasive tick species. This decision was made in part because the incidence of Lyme disease in humans continues to rise, and ticks infected with *Borrelia burgdorferi,* the pathogen responsible for Lyme disease in humans, are being collected in record numbers. In contrast, bed bugs are not known as vectors of disease agents and some adequate control options have already been developed to address bed bug infestations.

NP 104 is one of 16 national programs within ARS, and this NP 104 Action Plan, which extends from 2019-2024, will set important research priorities as ARS enters its eighth decade of existence. NP 104 research focuses on developing solutions for current agricultural problems and threats caused by arthropods of medical, veterinary, and urban importance in the United States, and on potential threats developing in other countries. Each problem statement will focus on addressing at least one of the four elements of the Integrated Pest Management (IPM) strategy discussed in more detail below.

Integrated Pest Management. Integrated Pest Management (IPM) is an ecologically-based approach to the management of pest populations. The approach was developed in response to overuse and overreliance on chemical pesticides and their declining effectiveness in reducing pest populations and associated diseases and damages. Effective IPM strategies can be classified into four elements: (1) risk assessment and biology; (2) surveillance; (3) control; and (4) monitoring and sustainability. These elements are listed in the order in which they would be applied operationally, *i.e.*, identifying the problem and threat, assessing the scope of the problem, mitigating the problem, and developing an effective long-term adaptable solution. Each element is discussed in more detail below.

Risk Assessment and Biology: Risk assessment and basic biological studies are critical first steps in the developing an IPM program. Defining the problem and selecting appropriate control strategies requires obtaining background information on pest identification (systematics and taxonomy), distribution (spatial and temporal), behavior (particularly behaviors that cause or have the potential to cause damage), genetics, and bionomics. Fundamental research on pest biology generates information that can be used to identify weaknesses of the pest; findings can also be used to help develop models that assess entomological and/or epidemiological risk to host populations. Studies focused upon the relationship between vector and pathogens fall under this element.

Surveillance: Surveillance provides assessments of the composition and abundance of pest populations. This information is needed to determine pest risk levels, identify when control measures are required, select appropriate control measures, and estimate the efficacy of alternative control strategies. Surveillance can also be conducted to determine the extent of pest damage; track infection levels associated with disease vectors; and assess environmental conditions, such as soil and moisture content, that affect larval habitats. Accurate surveillance data is critical to overall IPM programs, and research on developing efficient traps for collecting target pest species is an important component of this work.

Control: Pest control is a key outcome of IPM; it is a very broad category of activities that essentially consist of management tools, including conventional chemical controls, that prevent pests from accessing structures or hosts. These efforts often prevent or mitigate the harm pests can inflict on living organisms (e.g., disease transmission or injury from parasitic activity) and reduce pest damage to structures and associated developments. Effective pest control with a single intervention is often risky and unattainable, so multiple measures are often combined to achieve optimal success and minimize negative environmental effects. In some cases, control is obtained by suppressing pests that reach the target, and in other cases, control is achieved by killing the pest. Common control classifications include the following:

- Cultural control, *e.g.*, practices to reduce pest establishment, reproduction, dispersal, and survival;
- Physical control (source reduction and mechanical control), *e.g.*, reduction of breeding areas, barriers such as screen windows;
- Biological control (*e.g.* introducing specialist parasitic insects that feed on or otherwise inflect damage to invasive arthropods, plants, or pathogenic landscapes); and
- Chemical control using synthetic insecticides or bio-rational compounds (*e.g.*, botanical compounds, double-stranded RNA, etc.).

Genetic studies of pests are rapidly generating a wealth of information that can be used to develop new and adaptive pest control measures. In practice, each genetically-based control method is applied individually and locally, so it is especially challenging to project if or how laboratory results will be replicated in integrated field studies. Developing new genetic and chemical pest control measures that result in commercial IPM products often requires coordinating stakeholder efforts, funding, and other resources

Monitoring and Sustainability: Continuous monitoring of IPM performance is essential for assessing the efficacy of control interventions. Sustaining interventions can be challenging because they are often expensive and usually discontinued once success is achieved. However, pest populations that survive an initial IPM effort may develop resistance to chemical control methods, while pests may also be reintroduced after control efforts are no longer active, so sustained pest monitoring is necessary for early detection and rapid mitigation. Monitoring tools are similar to tools developed for pest, disease, and/or damage surveillance; however, deployment of these tools requires a more comprehensive strategy.

All research conducted within NP 104 supports one or more of the four elements of IPM as discussed, and the NP 104 research program covers the spectrum from fundamental to applied studies. Fundamental research provides information that identifies and explains previously unknown or misunderstood elements of pest biology, pest behavior, pest responses to control methodologies, and the impacts of control strategies on surrounding ecosystems. Translational research is also needed to develop or enhance management strategies or technologies that target known pest vulnerabilities. Finally, applied research is conducted to demonstrate the efficacy of strategies or technologies for pest control, or to demonstrate the level to which an integrated approach is achieving the overall goal of reducing pest populations and/or disease risk.

Research Component Overview:

The NP 104 Action Plan contains general strategies and specific actions within the following organizational hierarchy:

1) Components, which are general categories of research areas developed with input from stakeholders.
2) Problem Statements, indicating the specific nature and scope of problems to be solved by ARS.
3) Research Focus, which indicates the needs and the research that ARS will perform to solve the problem.

The components of the program have been modified to clarify the organization of the program relative to its name, National Program 104: Veterinary, Medical, and Urban Entomology. Additionally, the sole focus of the urban entomology program is invasive ants; therefore, the title of this component is named to better describe that focus.

The components of the program are:

Component 1: Veterinary Entomology
Component 2: Medical Entomology
Component 3: Fire Ants and other Invasive Ants

Component 1: Veterinary Entomology

Veterinary entomology research within NP 104 is comprised of research with a primary focus on arthropods that harm livestock and negatively impact animal agricultural production. The goal of this research is to protect animals from damage and diseases with an ultimate impact of improved animal health and well-being, increased productivity, and greater economic yields. There is a broad range of livestock and other animals that need protection from a vast range of arthropods and arthropod-borne diseases. Control of arthropods and reduction of disease risk to animals is a more complex problem than control of arthropods involved in purely anthroponotic cycles. Zoonotic cycles are more difficult to interrupt because animals tend to remain in the outdoor environment, providing arthropods with perpetual access to host animals. Animals are more susceptible to arthropod-borne infections and suffer direct damage from bites and infestations. In addition, there are arthropods that are not vectors of livestock pathogens or parasites, but still negatively impact productivity and economic yield due to stress from arthropod bites. When the disease risk is not present, it is common to accept a higher bite threshold for animals than the threshold set or tolerated by humans. Humans can convey their objection to nuisance biting in a direct manner compared to animals, where humans must interpret the nuisance from the animal's behavior. With respect to control methods and the use of conventional chemical control, animals are subjected to greater exposures of pesticides in the environment, and in some cases, there are pesticides used directly on animals to prevent arthropod attack. Animals are eaten as food; therefore, chemical residues within animal food products are of concern because they can accumulate in humans through consumption. There have been successes in the field of veterinary entomology, and some of these are the control of cattle lice, sheep mange mites, cattle bot flies, and the successful elimination of cattle fever ticks and screwworm flies from the United States. The latter two of these are still foci of ARS programs where continual effort is necessary to keep the United States free from these pests. There are many livestock pests which are not well controlled and some of these are addressed in this plan.

Based upon customer/stakeholder input, the arthropods of veterinary importance that are of greatest concern are: cattle fever ticks, screwworm flies, stable and horn flies, house flies, biting midges, and mosquitoes. Additional research on invasive ticks is added to address the recent expansion of Lyme disease and discovery that the East Asian tick is present in the United States.

Problem Statement 1A: Improved Integrated Pest Management of Ticks of Veterinary Importance. Ticks bite livestock and transmit pathogens that cause disease. *Babesia* pathogens transmitted by cattle fever ticks are of great concern because they cause bovine babesiosis, an expensive and potentially fatal disease in cattle. Strategies to better protect cattle and other livestock from the bites of ticks will lead to improved livestock health and production. Keeping cattle fever ticks outside of the United States is a national priority for the livestock industry.

Cattle fever ticks, *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus* can be infected with *Babesia* parasites and transmit these to cattle when they feed. Bovine babesiosis leads to production losses and death in cattle. The Cattle Fever Tick Eradication Program (CFTEP) was established in 1906 as the first U.S. livestock pest eradication program. Successful eradication of cattle fever ticks was accomplished in Texas by 1943, and in the 1960s for the rest of the United States. Since then, APHIS and the Texas Animal Health Commission maintain a quarantine zone in southeastern Texas where Mexican cattle, and wildlife frequently cross the international border with attached ticks. The mitigation strategies used within this zone continue to keep cattle fever ticks out of the United States, but sustainability is an issue due to expense of dipping infested cattle in acaricides, quarantining pastures, and by tick populations moving on much-expanded populations of native white-tailed deer and exotic ungulates (e.g., nilgai). NP 104 has made progress toward overcoming these challenges and needs to continue those efforts. Additional threats to livestock by high-consequence foreign pests are always present and one example of this is the recent discovery of the East Asian tick, *Haemaphysalis longicornis* in nine states: New Jersey, Virginia, West Virginia, Arkansas, North Carolina, New York, Pennsylvania, Maryland, and Connecticut. There are other potentially invasive tick vectors (*e.g., Amblyomma variegatum, Hyalomma* spp., *Rhipicephalus appendiculatus*) and species that might expand their ranges within the United States (e.g., *A. americanum, A. maculatum, A. mixtum*). Likewise, there are high-consequence foreign tick-borne pathogens, like African swine fever, that could devastate U.S. animal agriculture if their emergence involved transmission by native tick species.

Research Focus

Risk Assessment and Biology

- Assessment of ecological drivers for range expansion, fluctuation of cattle fever outbreaks in the United States, and potential introduction of invasive ticks to the United States.
- Identification of pathways for invasion by foreign ticks transported into the United States.
- Determination of pathogenic landscapes contributing to suitable tick habitats.

Surveillance

- Evaluation of strategies to improve detection of infestations with cattle fever and other ticks that eliminates or reduces the need for animal inspections.

Control

- Development of methods to mitigate impact of invasive weeds that facilitate cattle fever tick survival and reinvasion of the United States.
- Development of novel technologies for eradication and control of cattle fever ticks and other ticks, including formulations used against ticks which have developed resistance to current commercial acaricides.

Monitoring and Sustainability

- Development of improved methods to prevent introduction of exotic ticks.

Anticipated Products

- Decision-making tools to lessen the burden of ticks and the risk for tick-borne disease transmission.
- Sustainable technologies for control of cattle fever ticks and other invasive ticks.
- Countermeasures to mitigate the threat of invasive tick species and the diseases they can transmit.

Potential Benefits

- Improved surveillance and detection of invasive ticks.
- New control technologies for cattle fever ticks and other ticks infesting livestock and wildlife.
- Decreased economic losses due to bovine babesiosis.
- More effective prevention of cattle fever tick entry into the United States.

Problem Statement 1B: Improved Integrated Pest Management of Stable Flies that Feed on Livestock. Stable flies feed on livestock causing significant animal stress and loss of vigor leading to production losses, increased susceptibility to disease, and in extreme cases, death. Strategies to better protect livestock from stable flies and strategies to reduce stable fly populations will result in improved livestock health, production, and welfare.

Although stable flies are not normally considered a vector of disease for cattle and other livestock in the United States, their painful bites have direct impacts on cattle, such as reduced weight gain and in some cases death due to stress from the bites. Stable flies, native to southern Asia and Africa, were introduced to the United States with the first European colonists. The genus, *Stomoxys,* includes over twenty species in Africa and southern Asia. At least four species are pests of cattle. Stable flies have been implicated as mechanical vectors of several livestock pathogens including trypanosomes, equine infectious anemia and lumpy skin disease viruses, and anthrax outside of the United States. In recent years, stable flies developing in crop residues have produced serious “outbreaks” outside of the United States. The risks of introduction of exotic species of *Stomoxys, Stomoxys*-borne diseases or outbreaks resulting from changing agronomic practices to the United States are unknown. Controlling this fly has been challenging because the larvae develop in a broad range of habitats consisting of decaying vegetation, often contaminated with animal urine and feces. Adult stable flies can fly long distances, infesting livestock many miles from their developmental sites. Because stable flies feed on their hosts for only a short period of time, and usually on the lower legs, control techniques such as ear tags, topical applications of insecticides, residual insecticides, and traps have had limited success. Larval control options are limited to sanitation (removal of developmental substrates) and the treatment of developmental substrates with Insect Growth Regulators such as Cyromazine (a strategy discovered by ARS).

Research Focus

Risk Assessment and Biology

- Estimation of the risk of introduction of exotic species of stable flies and their potential ranges in the United States.
- Determination of environmental factors that lead to stable fly population increases and outbreaks.

Surveillance

- Development of new methods for adult surveillance control based on attractants and traps.

Control

- Development of new methods of bite prevention based on repellents.
- Development of new strategies for larval control based on development substrate, bacterial, microbial, and the biology of stable fly life stages.

Monitoring and Sustainability

- Evaluation of genetic approaches for use in sterile-insect technique and other control approaches.

Anticipated Products

- Improved traps for surveillance and control of adult stable flies.
- Long term, low maintenance attract-and-kill devices.
- New attractants and attractive surfaces or fabrics that increase efficacy of attract-and-kill devices.
- Long term, low maintenance attract-and-kill devices.
- Novel management tools for control of larval and adult stable flies.
- Novel control technologies discovered by mining the stable fly genome.

Potential Benefits

- Decreased stable fly populations.
- Increased animal health and well-being.

- Increased animal production.

Problem Statement 1C: Improved Integrated Pest Management of House Flies that Harm Livestock. House flies are ubiquitous pests of veterinary importance that can harbor and transmit pathogens to livestock. Because many of these pathogens are zoonotic to humans, flies are equally important to both preharvest and post-harvest food (food safety). Studies to better characterize the role of houseflies in disease transmission and strategies to better control houseflies and reduce disease incidence will result in improved livestock health and production.

Flies can cause significant animal stress due to their gregarious activities, congregating on animals to feed on secretions from eyes, nares, teats, and open wounds. Flies also associate with manure and refuse, and aggregate on feed boxes - providing a direct bridge between these environments and transmitting microbes in the process. These pests are known to be mechanical and/or amplifying vectors of pathogens and these flies are found often near livestock. Infections causing mastitis, pink eye, and other illnesses are due to bacterial transmission from house flies. Since flies sometimes travel great distances from larval sources, agricultural operations that produce them are blamed for flies affecting humans. Sanitation is usually a part of fly control, but farming operations often cannot remove the many larval sources associated with animal waste, spilled feed, etc. Traps, residual insecticides, attractive baits, and biological control are all helpful. Larval control remains largely experimental because the maggots reside below the surface of media. Basic research on fly-microbe interactions across life history-microbiome interaction is needed to improve risk assessment and inform novel control strategies that collectively result in reduced disease transmission.

<u>Research Focus</u>

Risk Assessment and Biology

- Evaluation of the economic impact of flies on agriculture.
- Assessment of the risk of bacterial transmission by studying the interactions of flies and bacteria, and the interaction of flies and microbes throughout life history.

Control

- Determination of factors that favor or limit the effectiveness of parasitoids for fly management, including temperature, substrates, and impacts of other fly management tools.
- Development of novel methods to control adult flies with emphasis on limited use of pesticides and resistance management.

Monitoring and Sustainability

- Evaluation of novel microbial methods for managing adult flies and their compatibility with other management components.

<u>Anticipated Products</u>

- Improved systems for house fly management based on trapping, source reduction and beneficial microbes.
- Better larval detection and control strategies based on larval-produced volatiles.
- Improved management strategies for confined livestock operations based upon

research determined risk that house flies pose in harboring and transmitting pathogenic bacteria.
- New targets for controlling larvae based upon improved understanding of utilization of microbes in the developmental substrate (e.g. manure).

Potential Benefits
- Decreased nuisance effects from houseflies.
- Decreased incidence of disease.

Problem Statement 1D: Improved Risk Assessment, Biology, and Control of Horn Flies. Horn flies take multiple bloodmeals from cattle and other animals per day. The bites cause discomfort to the animals, reduce productivity, and can result in secondary infections which damage leather quality. Strategies to better protect cattle and other animals from horn flies will result in improved animal health and decreased production losses.

Horn flies were first reported in the United States in 1887. A 12% decrease in average daily growth rate of nursing calves has been reported for cows that were not treated. Despite direct treatment of livestock and use of feed-through growth regulators, horn flies remain a major problem for producers in the United States and southern South America. Horn fly adults tend to remain on cattle and oviposit in freshly dropped feces, then returning to the cattle for feeding. The close proximity of larval and adult habitats may make this species particularly susceptible to genetic control. Successful replication of these genetic techniques could reduce pesticide use and make a major improvement in the economy of cattle production.

Research Focus

Risk Assessment and Biology
- Determination of the economic impact of horn flies in the United States.
- Determination of the genetic basis for variation in infestation levels among individual cattle.

Control
- Mining of the horn fly genome for development of biotechnology-enhanced sterile-insect technique approaches.
- Examination of horn fly biology to develop new control strategies, including interruption of pathogen transmission.
- Discovery of antigens for novel anti-fly vaccine formulations.

Anticipated Products
- Novel attractants and repellents for use in horn fly surveillance and control.
- Control technologies with new modes of action.
- Integrated approaches to manage insecticide-resistant horn fly populations.

Potential Benefits
- Decreased damage to cattle from horn fly bites.
- improved cattle health.

Problem Statement 1E: Improved Integrated Pest Management of the New World Screwworm. Screwworm flies infest animals by ovipositing their eggs in an animal wound. The larvae that develop from the eggs consume the host animal tissue as they develop, resulting in hide damage and death to the animals. Sterile insect technique (SIT) is relied upon to control screwworms and keep animals in the United States free from their attack. Improvements to the SIT program are needed to enhance sustainability of this program.

ARS developed the screwworm SIT technique and implemented it to control the New World Screwworm fly (*Cochliomyia hominivorax*). By the 1960s, this pest was eradicated from the southern United States, Mexico, and all of Central America by systematic application of the sterile-insect technique. Currently, the U.S. government (USDA APHIS) funds 90% and the Panamanian government funds 10% of a binational commission responsible for the production and distribution of millions of radiation-sterilized screwworm flies in eastern Panama. This activity forms a barrier that prevents reentry of the damaging species into Central and North America. In 2016, there was an infestation of screwworms found in Key Deer in the Lower Florida Keys. ARS cooperated with APHIS to successfully eradicate screwworms during this outbreak. One of the most successful entomological programs of all time, this operation continues to require technical support from ARS to address new problems and to reduce costs.

Research Focus

Risk Assessment and Biology

- Determination of the population genetic structure of screwworm populations in the Americans and in the Caribbean.

Control

- Mining of the screwworm genome for biotechnology-enhanced sterile insect technique and other approaches for optimal eradication.

Monitoring and Sustainability

- Improvement of rearing procedures to increase screwworm production efficiency.

Anticipated Products

- Genetic database of screwworm populations for areas where it remains endemic in the Americas and in the Caribbean.
- Next generation genetic systems for screwworm eradication.
- Decreased cost of rearing male screwworms for the SIT program.

Potential Benefits

- Determination of infestation source.
- Improved production of male screwworms.
- Reduced program cost.
- Continued reduction of screwworms from the Caribbean, Mexico, and other regions of the Americas.

Problem Statement 1F: Improved Integrated Pest Management of Mosquitoes of Veterinary Importance. Mosquitoes bite animals and transmit pathogens that can result in disease and death in animals. The impact of nuisance mosquitoes on farm production has not been assessed since the 1970s. Also, of great concern are invasive pathogens that have detrimental impacts once they become established in the United States. A greater understanding of the threats that the United States faces is required to assess risk and develop strategies to mitigate this threat.

West Nile virus entered the United States in 1999 and spready rapidly from the east coast to the west in about three years. The United States faces additional disease threats that have the potential to be a greater societal detriment than West Nile virus. Livestock and poultry are heavily exposed to mosquitoes but, with few exceptions, there is little understanding of their current impact. Emphasis of research is placed usually on disease rather than nuisance vectors; however, with the introduction of an invasive pathogen a nuisance mosquito may instantly become a disease vector. The potential for introduction of damaging invasive species is particularly broad due to globalization. Rift Valley fever and Japanese encephalitis are viruses that can be transmitted by many species including *Aedes taeniorhychus*, *Aedes vexans* and *Culex tarsalis*. Further studies are needed to define the risk to the United States based upon increased understanding of basic biology and to develop mitigation strategies for the potential vectors and for nuisance biting mosquitoes.

Research Focus

Risk Assessment and Biology

- Use of population-environment modeling to identify ecological determinants of the spatial and temporal ranges of subpopulations of pestiferous or disease-vector mosquito species.
- Determination of biological characteristics of pestiferous or disease vector mosquito species that are likely to harm animal production through bites or pathogen transmission.
- Determine impact of invasive species, such as *Culex coronator*, on native mosquito populations to investigate whether the invasive species pose more of a health risk than the native species.

Surveillance

- Evaluation of surveillance methods for use against pestiferous and disease vector mosquitoes.

Control

- Evaluation of management strategies for use against pestiferous and disease vector mosquitoes.

Anticipated Products

- Quantitative assessment of mosquito damage to U.S. agriculture.
- Predictive models that provide early warning of unusually large populations of mosquitoes and the environmental conditions which lead to increased vectorial capacity.
- Population distribution projections up to three decades into the future based upon

climate change models for mosquito populations.
- Novel mosquito surveillance and management tools.
- Improved control strategies specific to habitat, ecological region, and mosquito species that are present.

Potential Benefits
- Identification of disease threat potential for the United States.
- Improved control strategies for potential vectors of invasive pathogens.
- Improved protection from mosquito bites to improve animal production.

Problem Statement 1G: Improved Risk Assessment, Biology, and Control of Biting Midges of Veterinary Importance. Hematophagous *Culicoides* spp. biting midges are of great agricultural importance as pests of livestock, equine and wildlife, and as vectors of orbiviruses, orthobunyaviruses, and rhabdoviruses. Additional research on vector innate immunity, gut microbiome-arbovirus interactions, and arbovirus transmission zone surveillance is needed to reduce disease risk to livestock and wildlife.

Although *Culicoides* transmitted arboviruses can cause devastating disease in mammals, their infections in midges are almost always nonpathogenic and persist for the life of the midge. Relatively little is known about the balancing effects of *Culicoides* environmental habitat-derived microbiome on arbovirus survival in the midge, and how specific environmental conditions can influence transmission-competent midge populations.

Research Focus
Risk Assessment and Biology
- Correlation of midge surveillance to environmental conditions in arbovirus transmission zones.
- Characterization of biting midge developmental sites.
- Determination of the effects of larval substrate on gut microbiome of midges.

Control
- Development of larval habitat treatments for biting midge population management.

Anticipated Products
- Knowledge of biotic and abiotic characteristics of developmental sites can help in predicting location of midges and will inform larval control strategies.
- Novel control based on innate immunity mechanisms that can be targeted to either increase lethality of arboviruses to *Culicoides* midges or decrease arbovirus transmissibility by these vectors.
- New control products for larval habitat treatments to reduce biting midge populations.

Potential Benefits
- Reduced ability of *Culicoides* midges to maintain persistent, productive arbovirus infections, and target specific habitats in arbovirus transmission zones.
- Reduced attack rate of and virus transmission to livestock and wildlife.

Component 1 Resources

- Agroecosystem Management Research Unit, Lincoln, NE
- Arthropod-borne Animal Diseases Research Unit, Manhattan, KS
- European Biological Control Laboratory, Thessaloniki, Greece
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD
- Livestock Arthropod Pest Research Unit, Kerrville, TX
- Mosquito and Fly Research Unit, Gainesville, FL

Component 2: Medical Entomology

Medical entomology research in NP 104 is comprised of research with a primary focus on arthropods that harm humans and have the potential to cause disease as a result of their bites. The USDA has been involved in medical entomology and public health since its inception in 1862. Many of the public health approaches of arthropod control are adapted from arthropod control used in the agricultural sector. The USDA emphasis on medical entomology was likely at its most active point during World War II, when the Department of War requested the assistance of the USDA in developing repellents and chemicals to protect service personnel while on missions. NP 104 retains this medical entomology effort with a mission to protect both U.S. service personnel and U.S. civilians from arthropod attack, while at home and while abroad. Scientists within the program still work closely with the Department of Defense on technologies to protect the war-fighter, and with other government agencies, such as the U.S. Environmental Protection Agency, to develop products to protect consumers from arthropod attack.

Based upon customer/stakeholder input, the medically important arthropods of greatest concern are: mosquitoes, house flies, ticks, and sand flies. Based upon the increase in tick-borne Lyme disease, additional resources will be directed at tick research. As a result, sand fly research has been decreased and bed bug research eliminated from the NP 104 Action Plan.

Problem Statement 2A: Improved Integrated Pest Management of Mosquitoes of Medical Importance. Mosquitoes bite humans and transmit pathogens that cause disease and death. The vectors that transmit these pathogens are in the environment and development of increased insecticide resistance makes control approaches less effective. Improved surveillance, and novel, sustainable control is necessary to reduce risk to U.S. military and civilians at home and while traveling abroad.

Worldwide, mosquitoes are the insects that threaten human health most severely. The most important diseases caused by mosquito-borne pathogens are malaria, dengue, Japanese encephalitis, and yellow fever. There are about 3,400 species of mosquitoes described, but only 200-300 are important to human health. Most studies of mosquitoes are concentrated on the important vectors and the species that are easiest to manipulate in the laboratory, even though other species may also transmit pathogens, serve as enzootic vectors, or cause severe annoyance problems.

The recent introductions of West Nile virus and Zika virus into the United States reinforces the need to continue research on the arthropod vectors that transmit disease pathogens. Introduction of Rift Valley Fever into the United States remains a significant threat. This disease has devastating impacts on animals and humans. Additionally, the corresponding recent significant expansion of the range of *Aedes aegypti* back into areas that it was displaced in the United States exposes even more people to diseases recently introduced in the United States. A significant emerging threat to the United States is Rift Valley fever, a mosquito-borne viral hemorrhagic disease which has devastating impacts on animals and humans.

Anopheles mosquitoes transmit the parasites that cause malaria. The United States had severe problems with malaria through the 1920s and it is still a principal cause of morbidity and mortality throughout the tropics and in parts of temperate Asia. The importance of malaria prevention is increasing as the military shifts emphasis to Africa and eastern Asia. In addition, there is a threat of invasive *Anopheles* species that could increase the likelihood of malaria epidemics, as happened when *An. arabiensis* invaded Brazil in the 1930s and Egypt in the 1940s.

Other genera of mosquitoes also include important species. *Ae. aegypti* remains the principal vector of dengue virus worldwide. This species is also the vector of Chikungunya and Zika viruses, and Mayaro virus, a related pathogen of emerging significance. Many of the lessons learned on *Ae. albopictus* control apply to *Ae. aegypti. Culex tarsalis* is a major vector of viral encephalitides in the western United States and *Cx. pipiens/quinquefasciatus* is an important vector throughout the United States and worldwide. Many floodwater *Aedes* and *Psorophora* species, including *Ae. vexans* and *Psorophora columbiae*, are severe pests and occasional viral vectors. The threat of new invasive species is particularly severe for mosquitoes that develop in containers, like *Ae. notoscriptus* from Australia, or that transmit a virus, like *Cx. tritaeniorhynchus* as a vector of Japanese encephalitis virus.

Research Focus

Risk Assessment and Biology

- Evaluation of the risk and economic impact of mosquito-borne pathogens during the next three decades, both domestically and in potential foreign military theaters, considering both human and animal diseases, as well as native, invasive, and potentially invasive vector species.
- Identification of species specific microbiomes in mosquitoes and determine their effects on the vector competence (or vectorial capacity) of mosquitoes.

Surveillance

- Development of a standardized vector detection and surveillance system that is effective for all important groups of mosquitoes and that provides rapid detection of vector species and pathogens, including methods that employ genomics and proteomics.

Control

- Development of biological control using sterile insect technique against medically important biting insects.
- Development of integrated personal protection systems including new topical

repellents that use a lower percentage of active ingredients; spatial repellents that protect a room, a group of people, or an individual from point sources; and textile treatments that are alternatives to current pyrethroid impregnation.

- Development of new and improved tools for adult mosquito control including adulticides, textile treatments (bed nets, curtains, clothing, military field materials), and larvicides that overcome current limitations on use.
- Development and evaluation of devices, techniques, insecticides, or spatial repellents that prevent mosquitoes from being transported on vehicles, aircraft, or ships.
- Coordination with military partners to evaluate effectiveness and use of ARS-invented technology under deployment conditions.

Monitoring and Sustainability

- Determination of the effects of pathogens in mosquitoes on susceptibility to control and personal protection techniques with emphasis on animal and human diseases such as Rift Valley fever, and other arboviruses recently introduced into the United States such as dengue, chikungunya, and Zika viruses.
- Determination of resistance status and mechanisms of important vector and pest species to the main control chemicals (currently pyrethroids).

Anticipated Products

- Models developed to identify areas of the United States at high risk of introduction of invasive vector mosquitoes or invasive emerging mosquito-borne pathogens.
- Mosquito host range and resistance status maps of major vector and pest species in the United States and in foreign military theaters to enhance mitigation programs by public health agencies to control vector driven disease outbreaks.
- Knowledge regarding efficacy of novel and conventional control strategies specific to habitat, ecological region, and target insect populations relevant to U.S. military deployments.
- Improved surveillance technologies.
- Improved personal protection strategies.
- Bioinsecticides and biorepellents containing new natural active ingredient(s) for use in sustainable management and control of mosquitoes.
- New insecticides containing natural product synthetic analog active ingredients for use in the management and control of mosquito populations.
- Microbial bioinsecticides from bacterial and fungal isolates for use in the sustainable management and control of mosquito populations.
- Identification of U.S. military field materials capable of being treated at the factory with residual adulticides effective at reducing medically important mosquitoes across a range of environments relevant to U.S. military deployments.
- Spatial repellents to protect defined areas from key species of mosquitoes across a range of environments relevant to U.S. military deployments.
- Knowledge of efficacy of sterilization technique, rearing capacity, and survival, behavior, and competitiveness in laboratory and field studies for potential use of SIT against natural populations of medically important biting insects in the United States.

- Database (catalogue) of species specific molecular characterization for the rapid identification of mosquitoes and associated pathogens as part of surveillance programs and as an early warning system for detection of invasive species and pathogens of man and animals.
- Knowledge of distinct and shared microbiomes of mosquito vectors by comparing life stages, different physiological states, and pathogenic challenge to identify potential targets for management strategies.

Potential Benefits

- Earlier detection of invasive species; more effective IPM of vectors and pest mosquitoes.
- Improved integrated personal protection from arthropod attack.

Problem Statement 2B: Improved Integrated Pest Management of House Flies of Medical Importance. Due to their indiscriminate feeding habits, predilection for microbe-rich environments, and synanthropic behavior, house flies are cosmopolitan pests of medical importance. House flies have been shown to harbor and transmit hundreds of species of microbes, including those causing food-borne illnesses in humans, such as the pathogenic bacteria *Escherichia coli* O157:H7 and *Salmonella enteriditis*. Studies to better characterize the relationship between pathogens and adult flies are needed to develop effective strategies to reduce food-borne illnesses in humans.

"Filth flies" in the families Muscidae, Calliphoridae, and Sarcophagidae transmit food-borne pathogens like *Escherichia coli, Salmonella enteriditis,* and noroviruses. Recent work has shown that house flies (*Musca domestica*), at least, amplify some pathogens in their guts. As a result, flies can be a major cause of diarrheal disease in humans. Little information exists on how pathogenic bacteria multiply within and on house flies, knowledge of which would greatly improve our ability to limit their role as vectors of those pathogens. The military sees diarrheal disease as its third most important infectious disease threat and routinely controls flies to protect deployed personnel. Unfortunately, the state of the art for fly control is much less effective than for mosquitoes. The best strategy to separate flies from humans is uncertain, though dozens of different kinds of products are used in specific situations. Among the gaps in these techniques are effective larval and adult control, interruption of fly movement from larval sources to humans, effective and maintenance-free interventions, such as attract and kill devices, and insecticide resistance management.

Research Focus

Risk Assessment and Biology

- Quantification of the distribution, health threat, and associated costs of flies with respect to various populations (e.g., urban, agricultural, military) and projected three decades into the future, accounting for climate change.
- Evaluation of gaps in techniques available for integrated fly control, especially in a military setting and including establishment of realistic action thresholds.

- Assessment of house fly interactions with human pathogens including bacterial survival and transmission.
- Conduction of bioinformatic studies of the house fly genome and pathogen-induced transcriptomes.

Surveillance

- Development of improved traps and baits.

Control

- Development of biological control agents.

Monitoring and Sustainability

- Conduction of physiological and other research that supports development of effective resistance management.

Anticipated Products

- Risk assessment of house flies as reservoirs and transmitters of pathogens impacting human health.
- Novel control methods based on disrupting pathogen transmission by targeting of key defense genes used during fly-microbe interactions.
- Improved traps and baits for use in sensitive areas, such as in food service and medical facilities.

Potential Benefits

- Reduced prevalence of fly-transmitted diseases.
- Improved human health especially in areas where flies have access to animal or human waste.
- Increased food safety.
- Improved surveillance and management systems using knowledge gained in fly behavior and trap development studies.

Problem Statement 2C: Improved Integrated Pest Management of Sand Flies of Medical Importance. Sand flies bite humans and if infected can transmit a number of pathogens, such as Phlebovirus, Rift Valley Fever virus and *Leishmania* parasites, the latter of which leads to a disease called leishmaniasis. There are two forms of this disease, a cutaneous form that produces skin lesions and a visceral form which affects internal organs. Improved understanding of sand fly behavior in the ecosystem and novel control strategies are needed to reduce risk to U.S. military and civilians while traveling abroad.

Phlebotomine sand flies are a subfamily of Psychodidae that include the familiar and non-biting drain flies. Sand flies transmit pathogens causing leishmaniasis, bartonellosis, and sand fly fever. They can be infected with one of three serotypes of *Phlebovirus* (Naples, Sicilian, and Toscana viruses) which may be transmitted and cause Pappataci fever in human populations in the subtropical regions of the Eastern Hemisphere. Sand flies can also transmit another very dangerous *Phlebovirus*, Rift Valley fever virus, which threatens agriculture globally, putting national economies and domestic animal and human populations at elevated risk. Typical control techniques like fogging and residual treatments were effective at controlling these pests. For the

U.S. military, sand fly fever has been a major problem in particular regions, but most recently leishmaniasis was a major problem in Iraq and a threat in Afghanistan. Leishmaniasis also occurs in Africa and the Western Hemisphere, though it is a very rare disease in the United States. From the standpoint of NP 104, research on sand flies is conducted to protect U.S. military personnel when deployed overseas.

Research Focus

Risk Assessment and Biology

- Identification of ecological determinants of the ranges of subpopulations of known vectors of leishmaniasis to humans, for example, *Phlebotomus orientalis* based on population-environment modeling.

Surveillance

- Development and evaluation of attractant-based surveillance devices for sand flies that provide a practical alternative to carbon dioxide-baited traps.

Control

- Discovery of personal protection products (e.g., topical repellents, treated bed nets) against sand flies.
- Improvement of sand fly IPM programs by evaluation of alternative toxicants and other control strategies.

Anticipated Products

- Predictive models that provide early warning of unusually large populations of, for example, *Phlebotomus orientalis*.
- Control strategies with efficacy specific to habitat, ecological region, and target sand fly populations.

Potential Benefits

- Fewer cases of leishmaniasis and sand fly fever in the military.

Problem Statement 2D: Improved Surveillance and Control of Ticks of Medical Importance. Ticks bite humans and transmit pathogens that cause diseases such as Lyme disease and Rocky Mountain spotted fever. Lyme disease has been increasing over the past few decades and the *Ixodid* tick species responsible for infecting humans are widespread along the western coast and almost entire eastern half of the United States. Other tick genera, such as *Amblyomma*, transmit different human pathogens, and are also responsible for Mammalian Meat Allergy (MMA) also known as “alpha-gal,” as well as being the presumed vector of a poorly-defined illness named Southern Tick-Associated Rash Illness (STARI). Improved surveillance, control, and risk assessment strategies are needed to protect Americans from tick-borne disease.

Ticks transmit a wide variety of pathogens, many of which are associated with potentially fatal disease. Fortunately, most of the diseases are relatively uncommon in the United States, including human babesiosis, Rocky Mountain spotted fever, ehrlichiosis, anaplasmosis, and Colorado tick fever. The big exception is Lyme disease caused by bacteria and transmitted by *Ixodes pacificus* on the West Coast and *Ixodes scapularis* in much of the rest of the United States. Lyme disease affects at least 20,000 people per year concentrated in the Northeast and the northern Midwest, though the Centers for Disease Control considers the disease vastly underreported with closer to 300,000 people infected annually. The public health and medical research communities are actively engaged with the clinical aspects of Lyme disease, but vector control and bite prevention are areas in which NP 104 has particular expertise. In addition, the Lone Star tick *Amblyomma americanum* is assuming importance because of its geographic range, pathogen transmission and its incrimination in other tick maladies. It is necessary to focus the program to the area in which NP 104 can make the greatest contribution rather than to address the full spectrum of problems. These problems include the challenge of preventing tick bites by personal protection, particularly when a high percentage of the bites are infected, and the tick is rapidly expanding its geographic range. Therefore, it is important to document the geographical range of a potential vector and conduct a risk assessment of the importation threat for exotic ticks originating from Europe, Asia, and Oceania.

Research Focus

Surveillance

- Development and evaluation of new techniques for surveillance and control of *Ixodes pacificus, Ixodes scapularis,* and *Amblyomma americanum;* and assemble available techniques into designs for integrated community control programs.

Control

- Discovery of antigens that can be used in anti-tick vaccines for use in wildlife against *Ixodes* ticks.
- Assessment of the suitability of anti-tick vaccines for potential use in deer against *Ixodes* ticks.

Anticipated Products

- Increased understanding of the role of individual tick species in disease transmission.
- Tools, including chemicals, to prevent tick bites.
- Novel vaccine formulations to control *Ixodes* ticks.
- IPM of deer (black-legged) ticks to prevent Lyme disease in suburban settings.

Potential Benefits

- Increased ability to control ticks.
- Fewer cases of Lyme disease and other tick-associated maladies in the United States.

Component 2 Resources

- Agroecosystem Management Research Unit, Lincoln, NE
- Arthropod-borne Animal Diseases Research Unit, Manhattan, KS
- European Biological Control Laboratory, Thessaloniki, Greece
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD
- Livestock Arthropod Pest Research Unit, Kerrville, TX
- Mosquito and Fly Research Unit, Gainesville, FL
- Natural Products Utilization Research Unit, Oxford, MS

Component 3: Fire Ants and other Invasive Ants

This NP 104 Component is comprised of research with an exclusive focus upon ants that harm humans, livestock, crops, and structures. Pest ants cause severe damage in agricultural and urban sectors and concurrently negatively impact natural environments. Most pest ants are invasive species. In the agricultural sector, ants have been shown to reduce crop yields and harm livestock by attacking small or newborn farm animals, like chicks or calves. Such attacks lead to physical damage, increased stress, and the potential death of these animals. Numerous high-value crop plants have been directly affected by ant feeding including soybeans, corn, okra, potatoes, almonds and citrus. Even farm equipment and irrigation systems have been damaged by pest ant species. The predatory nature of ants may also harm plants or crop production by reducing the density of pollinators and other beneficial insects, such as natural enemies. Certain species increase the densities of sap-sucking pest insects (aphids and coccids) that transmit diseases directly or encourage growth of molds on important crops such as grapes, citrus and cucurbits. Pest ant-contaminated shipments into domestic or foreign controlled areas impede commerce when they are subject to rejection and returned to their point of origin. This is of high concern in the plant nursery, sod, and forage industries. Ants also are the most common arthropod pests in urban environments and account for more complaints to pest control companies than any other insect group. Some ant species sting and are known to cause anaphylaxis and even death. In natural environments, invasive ants often displace native ant fauna, reduce arthropod biodiversity, and potentially disrupt entire ecological communities as invading “ecosystem engineers” due to their dominance and the varied functional roles they have in ecosystems.

Based upon customer/stakeholder input, the ant species of greatest concern are: imported fire ants, Argentine ants, crazy ants, electric ants (little fire ants), carpenter ants, Asian needle ants, white-footed ants, odorous house ants, dark rover ants, Pharaoh ants, and big-headed ants.

Problem Statement 3A: Improved Risk Assessment, Biology, and Control of Invasive Fire Ants.
Invasive fire ants harm humans, animals, and cause significant economic losses from damage to crops and structures. Sustainable, affordable approaches to long term suppression of populations do not exist. Improved and novel control interventions and approaches are needed to control this pest.

Fire ants are considered major agricultural, medical, urban, and environmental pests in the United States. Imported fire ants currently infest over 365 million acres from Virginia south to Florida and west to California. Beyond the continental United States, these invasive ants have expanded into Puerto Rico, and most of the other Caribbean islands, Australia, Taiwan, China and India. Agricultural, economic and medical costs due to fire ants exceed $6 billion dollars annually in the United States and commercial chemical treatments for control are estimated to cost as much as $40 per acre. The affected urban and agricultural sectors are broad in range and include households, schools and recreation areas, electric and communication equipment, and animal and plant agriculture. Fire ant control depends heavily on the use of synthetic insecticides. There are two major chemical methods available to control the red imported fire ant: baits using slow-acting insecticides and spray/drench/granule mound treatment using fast-acting contact insecticide. Both methods have problems. Current fire ant bait products lack specificity (harm to native ants) and are sensitive to water. For mound treatment, the major problem is the potential impact of synthetic insecticides on the environment. Both methods provide only temporary fire ant suppression. There is a need to develop new inexpensive, environmentally friendly, and fire ant-specific products and their delivery techniques. More than 80 years after its introduction into the United States, the red imported fire ant is still spreading. Novel detection methods to facilitate interception of fire ants at quarantine boundaries are needed to reduce continued spread of fire ants.

A solution for alleviating the heavy dependence on synthetic insecticide is to implement biological control and develop biopesticides in fire ant management. Intercontinental comparisons between native (South America) and introduced (United States) fire ant populations have shown significant differences in the biology, genetics, and reproductive behavior of ants from the two areas. These comparative studies have also shown that numerous natural enemies (parasitoids, parasites, and pathogens) are associated with fire ants in South America, but only a few of these have been found from invasive fire ants in the United States. Extensive integrated studies are needed to understand biological differences between native and introduced populations and to identify the most appropriate natural enemies to release for sustainable biological control of fire ants.

Many natural materials have been reported to be toxic and/or repellant to fire ants such as citrus oil, mint oil, essential oil from the leaf of *Cinnamomum osmophloeum* Kaneh, sweet wormwood oil, Nootka oil, and Sweet Orange essential oil. Recently several naturally occurring benzoate analogs have been found by ARS scientists to be potent toxins against fire ants and one such benzoate analog has neither known OSHA hazards nor aquatic toxicity indicating a great potential for their application in fire ant management. Natural products may be a promising source of new active ingredients for development of environmentally friendly fire ant control products. Interestingly, the ARS research also showed that some environmentally benign synthetic compounds are also potent fire ant toxins. These compounds may be also a source of safe active ingredients for fire ant control.

High selectivity is a desirable property for an ant control product. However, most active ingredients used in current ant control products are broad-spectrum insecticides that affect non-target ants and other insects. Genetic control strategies hold powerful potential for very specific intervention against invasive ants. Because gene sequences are extraordinarily unique, a gene disruption product hypothetically can target not only a single species, but a specific population within a species. Previous studies show a given ant species harbors hundreds to thousands of unique genes not found in other ants. A control product based on invasive ant genes will benefit the entire ecosystem disturbed by invasive species.

<u>Research Focus</u>

Risk Assessment and Biology

- Investigation of the ecological, behavioral, and competitive interactions of invasive pest ants that are sympatric with fire ants, e.g., the tawny crazy ant and the big-headed ant.
- Development of methods capable of identifying numerous fire ant species.

- Identification of essential molecular and/or receptor antagonist/agonists targets through molecular and metabolomic research.

Control

- Discovery, collection, and evaluation of new natural enemies (parasitoids, parasites, and pathogens) of native South American fire ants for potential use as biological control agents of introduced fire ants in the United States.
- Improvement in virulence of fire ant pathogens by selection of strains in ants and *in vitro*.
- Design and evaluation of bait and mound treatment formulations with pathogens, natural products, and environmentally friendly synthetic chemical compounds as the active ingredients.
- Investigation of novel approaches to fire ant-specific control, including new active ingredients (oil and water soluble), and attractants and phagostimulants for bait enhancement.
- Investigation of repellents and anti-phagostimulants including those derived from plants, that exclude the ants from specific areas, and/or protect domestic and livestock food materials.
- Determination of gene function and utilize existing genomic resources to develop fire ant-specific control methods and products.
- Development of new and improved insecticide delivery technologies to reduce the use of synthetic insecticide in fire ant management.

Anticipated Products

- Knowledge of the dynamics of pest ants that are sympatric with invasive fire ants.
- Field-ready rapid method to detect and discriminate red and black imported fire ants from other ant species.
- Classical biological control agents capable of providing sustained control of fire ants.
- Formulations capable of excluding the fire ant from areas designated as free zones.
- Novel fire ant control based on new biochemical and molecular targets.
- Novel and improved methods to deter fire ant invasion, spread, and methods and products to mitigate infestations and damage.

Potential Benefits

- Improved understanding of ants and their impact.
- Improved sustainable fire ant control throughout the United States.

Problem Statement 3B: Improved Integrated Pest Management of Invasive Crazy Ants. Invasive crazy ants impact agriculture and human activities. This species was introduced into the United States about fifteen years ago and therefore little is known about the biology of this ant species. Basic biological studies along with new and improved control approaches are needed to achieve adequate control**.**

The tawny crazy ant, *Nylanderia fulva*, is an invasive ant from South America that develops extremely dense populations in the United States, which inundate and dominate natural, agricultural, and urban landscapes. Tawny crazy ants currently infest at least 28 counties in Florida, 29 counties in Texas, the island of St. Croix (U.S. Virgin Islands), and is spreading into Georgia, Alabama, Louisiana, and Mississippi. Tawny crazy ants impact agriculture by feeding on plant carbohydrate sources, they tend and protect pathogen-transmitting aphids, asphyxiate small livestock, e.g., chickens, and stress large livestock. They also affect natural resources, recreational areas, and residential environments by, endangering wildlife, reducing biodiversity, infiltrating buildings, and decreasing value and enjoyment of infested lands due to relentless numbers of ants. In addition, high densities of these ants have resulted in excessive pesticide use, which poses a serious hazard to humans and the environment. Control options for tawny crazy ants are severely limited, mainly dependent on residual insecticide sprays that are problematic in areas like nature preserves where non-target damage to insects is an issue. IPM strategies based on biology, baiting, and biological control are needed to obtain near and long-term control of invasive tawny crazy ants.

Research Focus

Risk Assessment and Biology

- Evaluation of the relationships between tawny crazy ants and other ant species through basic ecological and biological research.

Surveillance

- Investigation of the basic behavioral and reproduction ecology (biology) and chemical ecology of tawny crazy ants to improve surveillance.

Control

- Investigation of the basic behavioral and reproduction ecology (biology) and chemical ecology of tawny crazy ants to improve bait specificity and control.
- Discovery of natural enemies of tawny crazy ants suitable as biological control agents.
- Identification of effective bait active ingredients and formulations for tawny crazy ant control.
- Development of effective baiting strategies for tawny crazy ants such as timing bait applications relative to the seasonal phenology of brood production.
- Establishment of genetic resources as a foundation for developing next-generation genetic control for crazy ants.

Anticipated Products

- An understanding of how tawny crazy ant colonies grow, mature, and decline or migrate.
- An understanding of the dynamic interactions of sympatric pest ant species.
- Surveillance tools and improved bait specificity.
- Natural enemies for classical biological control (natural) of Tawny crazy ants.
- Effective bait(s) for tawny crazy ant control.

Potential Benefits

- Improved understanding of tawny crazy ants and their impact.
- Improved and sustainable control of *Nylanderia* crazy ants.

Problem Statement 3C: Improved Integrated Pest Management of Other Invasive Pest Ants. Other invasive pest ant species exist that harm humans and animals, and damage crops and structures. The United States remains at risk of introduction of invasive species. Basic biological studies, including improved surveillance, control, and understanding the ecosystem that the invasive ants thrive in are needed to better protect the United States from invasive and native ants.

Numerous additional ant species are also serious pests of agriculture, residences, and the natural environment, including: electric ants (little fire ants), Argentine ants, big-headed ants, white-footed ants, Pharaoh ants, and Asian needle ants. Many of the technologies developed for imported fire ants and tawny crazy ants may be adapted for use with these and other pest ants. A better understanding of the climatic and habitat requirements (applied ecology) of these pest ants will lead to improved predictions of their potential range and where they will cause the most problems.

Research Focus

Risk Assessment and Biology

- Development of models to predict the invasive ants likely to become future pests in the United States and to predict future range expansions of invasive ants already present in the United States.
- Expansion of the genetic understanding of invasive ants, individually and comparatively, through sequencing and recombinant technologies.
- Investigation of potential impacts of abiotic factors on invasive and native pest ants.
- Determination of comparative invasive pest ant characteristics, *e.g.,* competitiveness, and reproductive biology through research on the basic biology and ecology of selected pest ants.

Surveillance

- Examination of chemical ecology and semiochemical approaches to enhance bait effectiveness and improve surveillance and detection methods for select pest ants.

Control

- Investigation of the chemical ecology and semiochemical approaches of invasive ants to improve bait specificity and control for select pest ants.

Anticipated Products

- An understanding of intraspecific and interspecific colony dynamics.
- Improved surveillance methods.
- Ant bait formulation(s) effective against selected invasive ant species.
- Natural enemies for classical biological control of an invasive ant species.
- New pest management and IPM strategies for selected invasive ant species.

Potential Benefits

- Improved understanding of invasive ants and their impact.
- Assessment of threat from invasive ants and development of management tools to control ants.

Component 3 Resources

- Biological Control of Pests Research Unit, Stoneville, MS
- Imported Fire Ant and Household Insect Research Unit, Gainesville, FL
- Natural Products Utilization Research Unit, Oxford, MS