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National Program 104

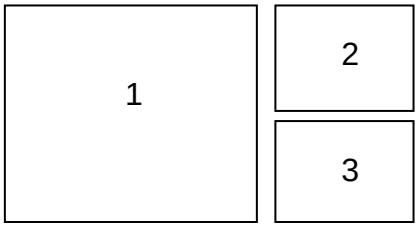
Veterinary, Medical, and Urban Entomology

Accomplishment Report 2007-2011

2 ... 4 ... 6 ... 8

Protecting two- and four-legged animals from six- and eight-legged arthropods

Captions of front page photos:



1. A male deer eats grain at a 4-Poster Deer Treatment Bait Station developed by ARS scientists that uses rollers to apply acaricide to the head of the animal.

2. The stable fly, *Stomoxys calcitrans.*

3. The yellow fever mosquito, *Aedes aegypti.*

National Program 104
Veterinary, Medical, and Urban Entomology

ACCOMPLISHMENT REPORT 2007-2011

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The National Program 104 Action Plan 2009-2013
Components and Problem Statements

Component 1: Medical Entomology for the Public

PROBLEM STATEMENT 1A: *Priority species of mosquito (e.g.,* Aedes aegypti *and* Aedes albopictus*, floodwater mosquitoes,* Culex *vectors of viruses, and* Anopheles*) are often controlled inefficiently because appropriate tools either do not exist or are not used in the most effective manner.*

PROBLEM STATEMENT 1B: *Tick pests of humans (e.g.,* Ixodes scapularis *and* Ixodes pacificus *[Lyme disease];* Dermacentor variabilis *and* andersoni *[Rocky Mountain Spotted Fever];* Amblyomma americanum *[Human Monocytic Ehrlichiosis]) are often controlled inefficiently because appropriate tools either do not exist or are not used in the most effective manner.*

PROBLEM STATEMENT 1C: *Flies, as mechanical vectors of pathogens to humans and food, are generally controlled poorly, resulting in standing populations capable of inoculating food and wounds with pathogens.*

PROBLEM STATEMENT 1D: *Bed bugs have become much more abundant in the United States during the last five years, presenting the need for better surveillance and control technology.*

PROBLEM STATEMENT 1E: *Invasive vectors of human pathogens should be detected early, stopped before they can establish themselves, or eradicated if they become established.*

Component 2: Medical Entomology for the Military

PROBLEM STATEMENT 2A: *Toxicants currently available for controlling arthropod vectors are not adequate to protect deployed military personnel. The development of public health pesticides does not receive as much attention from industry as the development of agricultural pesticides. As a result, the number of active ingredients is limited and important pests are not listed on the labels of existing products. The situation is dangerous because loss of any of the current active ingredients to resistance or environmental concern could lead to an inability to control some vectors. Recent failure by the military to control sand-fly transmitted leishmaniasis in Iraq was a consequence of the absence of a suitable toxicant.*

PROBLEM STATEMENT 2B: *Personal protection products against arthropod vectors are not capable of providing optimal protection for military personnel. The current military system for personal protection consists of the Extended Duration Topical Insect and Arthropod Repellent (EDTIAR), permethrin treatment of uniforms, and permethrin treatment of bed nets. Each component of this system has serious problems. The EDTIAR*

is thick and difficult to apply, in part because the 8-12 hour efficacy required necessitates a high percentage of the active ingredient (deet). Permethrin treatment of uniforms only protects the skin it covers, offering little protection by itself against flying vectors like mosquitoes. Also, permethrin treatment continues to present logistical challenges despite almost 20 years of experience with this technology. Finally, it has been difficult to develop a rugged design for bed nets that is compact, easy to use, and pre-treated with a chemical to prevent biting through the mesh.

PROBLEM STATEMENT 2C: *Application techniques and equipment for dispersal of pesticides are not adequate to protect deployed military personnel from pathogens transmitted by arthropods. Identification of an effective active ingredient is only part of what is needed for a good insecticidal treatment. Formulation of the active ingredient imparts characteristics necessary for a particular use. For example, good spreading characteristics and long residual would be desirable for a barrier treatment applied to vegetation. The equipment dispensing the pesticide is also important, since it controls many factors that affect final distribution of the toxicant.*

Component 3: Veterinary Entomology of Livestock and Poultry

PROBLEM STATEMENT 3A: *The* Rhipicephalus (Boophilus) *one-host tick transmits bovine babesiosis (Texas cattle fever) among cattle. An extensive effort started in the early 1900s culminated in eradication of this tick from the entire U.S. in 1943, eliminating the threat of Texas cattle fever to the beef industry. In recent years, the cattle fever tick has been expanding its range into the southern counties of Texas despite the continuing efforts by APHIS (Cattle Fever Tick Eradication Program) to maintain the control area. The expansion in range is probably the result of the increase in numbers of an alternative host, the white-tailed deer. In addition, feral exotic ungulates (e.g., axis deer) may contribute to the tick infestations. At the current time, the dollar value of the ungulate hunting business is greater than the value of cattle in parts of this region, making landowners reluctant to limit the ungulate herds. Although laboratory tests indicate that introduced ticks are usually susceptible to acaricides, dipping treatments of infested cattle herds seem to be failing more often. Unless this situation is understood and controlled, the cattle industry throughout the southern United States could be threatened with a disease that has been controlled for over 60 years.*

PROBLEM STATEMENT 3B: *Stable flies (*Stomoxys *species) are not adequately controlled on most farms and there is a threat of importation of other, related species of flies.*

PROBLEM STATEMENT 3C: *The house fly (*Musca domestica*) is not adequately controlled on most farms.*

PROBLEM STATEMENT 3D: *The horn fly (*Haematobia irritans*) can be a damaging pest of cattle in the United States.*

PROBLEM STATEMENT 3E: *The screwworm fly (*Cochliomyia hominivorax*) has been eliminated from North America thanks to a program of detection, animal treatment, and mass release of sterile male flies. The current status of APHIS' Screwworm Eradication Program is maintenance of a barrier zone in eastern Panama, protecting the entire continent to the north. This program involves a continuous obligation of funding in order to maintain the barrier and protect the U.S. from this devastating pest of mammals. Expansion of the program to the Caribbean and South America is a possibility, but not currently planned or funded by the U.S. government. Various governments and NGOs discuss such expansion and have funded several efforts. ARS continues to perform research that improves the efficiency of the rearing, release, and control of screwworm flies.*

PROBLEM STATEMENT 3F: *Mosquitoes bite domestic animals, but little is known about their potential to cause decreases in yield; control is difficult in rural settings.*

PROBLEM STATEMENT 3G: *Biting midges transmit viruses to cattle, sheep, and deer; the midges are often numerous enough to create a significant annoyance.*

PROBLEM STATEMENT 3H: *Phlebotomine sand flies transmit leishmaniasis and sand fly fever virus; current methods for control are not uniformly effective.*

PROBLEM STATEMENT 3I*: Imported and established in the United States, invasive vectors of livestock pathogens could threaten the industry by transmitting new pathogens.*

Component 4: Pests that Damage Structures

PROBLEM STATEMENT 4A: *Following the development of effective tools, the challenge to controlling FST is organization, creative technique, and application.*

PROBLEM STATEMENT 4B: *Invasive and native termites are a threat to structures*

Component 5: Fire Ants, other Invasive Ants, and Household Pests

PROBLEM STATEMENT 5A: *Imported fire ants threaten the safety of people on their property, the well-being of farm animals, and the condition of pastures.*

PROBLEM STATEMENT 5B: *Other invasive ants in the United States (e.g., Argentine ant, little fire ant, crazy ants, the tropical fire ant, white-footed ant, and carpenter ants) are either difficult to control or threaten to become established pests in the United States.*

PROBLEM STATEMENT 5C. *Potentially invasive ants (e.g. leaf-cutting ants and other fire ant species) could be introduced to the country, complicating already difficult ant control.*

PROBLEM STATEMENT 5D: *Emerging household pest insects could become established as major problems in the United States.*

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National Program 104
Veterinary, Medical and Urban Entomology

ACCOMPLISHMENT REPORT 2007-2011

BACKGROUND AND GENERAL INFORMATION

National Program 104: Veterinary, Medical and Urban Entomology (NP 104) ultimately aims to develop more effective methods of preventing or suppressing insects, ticks, and mites that affect animal and human well-being. Our logo is "*2-4-6-8—We protect the two- and four-legged from the six- and eight-legged.*"

The research conducted by the scientists within NP 104 focuses on producing solutions to problems in animal agriculture and public health created by arthropods. Those problems range from the discomfort created by biting insects and ticks to serious diseases caused by arthropod-transmitted pathogens. Ants are included in the program because of the effects of their bites on animals and people, damage to pastures and crops, and their status as household pests. Termites were an area of focus within NP 104, but that research was terminated during this reporting period.

As this report documents for 2007-2011, NP 104 has made significant contributions to integrated pest management (IPM) and biology of these types of arthropod pests. Progress has been made on development of new insect repellents, new insecticides, and new insect traps. The applied research of our scientists has lead to effective strategies for the integrated control of the Asian tiger mosquito, sand flies, biting midges, mosquitoes, and ticks. NP 104 scientists continue to perform research and development that improves the efficiency and effectiveness of large national efforts for the biological control of imported fire ants, the cattle fever tick eradication program, and maintenance of the barrier against reinvasion of Central and North America by the screwworm fly. Basic research by the USDA Agricultural Research Service (ARS) on chemical ecology, odorant reception, population genetics, genomics, microbial ecology, and insect physiology contributes to the field of science and provides raw material for future practical applications.

ARS scientists have developed a number of new products that have advanced the effectiveness of insect control, including products that have contributed to the ability to control the Formosan

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subterranean termite on an areawide basis, thus saving the French Quarter of New Orleans from destruction. They have also increased the reliability of protection of deployed military personnel from diseases transmitted by insects—developments that are also helpful for the civilian population. Biological controls developed by ARS continue to reduce the severity of the fire ant problem in the United States. New chemicals either extracted from nature or synthesized in the laboratory offer the promise of safer and more effective control of bed bugs, mosquitoes, and other public health and veterinary pests. Extensive cooperation with industry and energetic efforts to work with the Environmental Protection Agency (EPA) through the IR-4 program has pushed products toward the marketplace.

NP 104 consists of 12 active projects in 9 locations. The 38 scientists working in NP 104 form a multi-disciplinary group, trained in entomology, molecular biology, chemistry, global information systems, microbiology, and veterinary medicine.

PLANNING AND COORDINATION FOR THE NP 104 5-YEAR CYCLE

The standard organization of ARS national programs is hierarchical by component, problem, and anticipated product. The Action Plan that describes these goals was written in 2008 following considerable input from ARS scientists and a broad spectrum of stakeholders from agriculture, industry, government, and academia provided at a stakeholders' workshop in Denver in October 2007. Although the proposed research was organized within five components (military medical entomology, civilian medical entomology, veterinary entomology, termites, and ants), the Action Plan described four cross-cutting areas of emphasis identified by stakeholders:

- The application of spatial analysis and global information systems;
- The study and development of products from chemical ecology;
- The need for much better methods to control filth flies; and
- Attention to current and potential invasive species.

The Action Plan was used by the NP 104 National Program Leader (NPL) as the basis for assigning objectives to each of the research projects. In close coordination with research leaders and scientists, the NPL helped individual scientists develop objectives to each project. Lead scientists then wrote a preliminary project plan outline that described sub-objectives and outlined methods for a full project plan. The project plan outline allowed the NPL and researchers in the field to ensure that the projected work was relevant to the Action Plan, feasible, and likely to be productive. The lead scientists then wrote the project plans—documents that have a core text of some 25 pages and a level of detail similar to that of a grant proposal. The plans were reviewed by an external peer panel coordinated by the ARS Office of Scientific Quality Review for relevancy and scientific quality. The peer review panels made suggestions for modification of some project plans, which were addressed by the lead scientists and subsequently approved by the panel.

The next 5-year research cycle for NP 104 will begin with a new Action Plan, to be written between March and May of 2013, and new project plans to be developed in the early part of 2014.

STRUCTURE OF NATIONAL PROGRAM 104

The projects within NP 104 are assigned to one of five components specifically targeting research for a particular type of insect.

Component 1: Medical entomology for the public
ARS has developed expertise in safe insect control for over a century. As a result, ARS research is in demand for development of methods to protect people as well as animals from damage caused by insect bites and from diseases transmitted by arthropods. Many of the techniques developed for protection of food animals have application for protection of people. ARS scientists who are experts in medical and veterinary entomology are able to recognize developments that may have application to either discipline, as well as perform research directly relevant to these problems.

Component 2: Military medical entomology
These efforts are intended to satisfy the need for the military to protect Americans anywhere in the world from diseases transmitted by insects. The problem is particularly important for the United States because most military personnel have had no exposure to the pathogens to which they might be exposed during a deployment. Recent military successes have been dependent on keeping people healthy despite challenging environments that include threats from malaria, dengue, and other diseases caused by arthropod-borne pathogens. The Deployed Warfighter Protection Program (DWFP) provides supplemental funding from the Department of Defense (DoD) to help ARS accomplish these goals.

Component 3: Veterinary entomology of livestock and poultry
The scientists working on research in this component are focused on cattle fever ticks and screwworm flies, pests that have been eradicated from the United States, but that require constant operational activity to maintain the success. Other pests also under research and considered the most important current threats to U.S. animal agriculture include stable flies, house flies, horn flies, mosquitoes, biting midges, phlebotomine sand flies, and vectors that are actual or potential invasive species.

Component 4: Pests that damage structures
The research in this Component was targeted at the Formosan subterranean termite in response to a general threat to structures throughout the Southeast and a particular threat to the French Quarter of New Orleans, Louisiana. The research was performed in-house and also by a group of cooperators throughout the Southeast and in Hawaii.

Component 5: Fire ants, other invasive ants, and household pests
Current research efforts within this Component are concentrated on ants, which have both urban and agricultural significance. The bulk of the work has been directed at the red imported fire ant and somewhat less at the black imported fire ant, although the current Action Plan expands the effort to other invasive ant species. Those ants affect agriculture in many ways, including disruption of ecological services, destruction of pasture, direct damage to livestock, and direct damage to crops such as grapes.

Within the five components, this National Program has attempted to order research around IPM defined functionally in four roughly sequential steps:

1. **Risk assessment and biology:** Finding the problem and establishing relevant background.
2. **Surveillance:** Determining location and abundance.
3. **Control:** Suite of methods used in the most efficient combination and in a way that causes no harm.
4. **Monitoring and sustainability:** Maintaining a successful outcome through vigilance and communication, including assurance of resource availability.

The structure of NP 104 has been significantly affected by budget cuts since the Action Plan was written. In 2011, Congress approved the cancellation of all earmarks and the closing of laboratories in 2012. These budget reductions came during the middle of the 5-year course of the Action Plan but, for the most part, after the period covered by this retrospective report.

NP 104 started the 5-year Action Plan period with 18 projects and will be finishing the course with 11 projects. The areas of research eliminated in this process were termite and West Nile virus studies, and Lyme disease work also was sharply reduced. It should be pointed out that the termite work had largely accomplished its goal of developing practical methods for areawide control of the Formosan subterranean termite, providing the means to save the French Quarter of New Orleans from destruction by this pest.

Briefly, earmark cuts resulted in the consolidation of two projects in Gainesville, Florida, (one on surveillance and the other on control of mosquitoes and biting flies) into a single project on IPM. Other terminations included a Lyme disease control project conducted at Yale University, research on West Nile virus transmission by the Connecticut Agricultural Experiment Station and the ARS laboratory in Gainesville, and a program in New Orleans funding university and ARS research on the Formosan subterranean termite and Hawaiian termites. The laboratory closures in 2012 eliminated ARS in-house termite projects in New Orleans, which was justified by the successful IPM of the Formosan subterranean termite in the French Quarter.

RELATIONSHIP OF NP 104 TO OTHER NATIONAL PROGRAMS

NP 104 has its closest relationships with National Program 103, Animal Health, which conducts research on animal diseases vectored by insects, and with National Program 304, Crop Protection and Quarantine, which conducts research on managing pest populations to protect crops.

The relationship between programs is particularly close at the Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas. Most of the scientists there work on both NP 103 and NP 104 projects, coordinating closely on work on diagnostics, vaccines, pathology, vector surveillance, physiology of pathogen transmission, and vector control.

The funds originating from DoD for the DWFP are mostly directed at NP 104 programs, but in order to accomplish its goals, the NPL for NP 104 directs some of the funding to the Natural Products Utilization Research Unit in Oxford, Mississippi (which does research in National Program 214, Agricultural and Industrial Byproducts), the Areawide Pest Management Research

Unit in College Station, Texas (which does research in National Program 305, Crop Production), and to an agreement with the University of Florida to fund a post-doctoral researcher at the European Biological Control Laboratory's satellite laboratory in Thessaloniki, Greece. DWFP funds are also used to support a full-time public health entomologist at the IR-4 Project at Rutgers University for the purpose of promoting registration of new public health pesticides. By bringing in a wider variety of skills than represented solely within NP 104 projects, ARS has created a virtual development laboratory that accomplishes most of the functions of a major pesticide development firm at a fraction of the cost.

Other collaboration across National Programs has resulted in sharing human and laboratory resources belonging to NP 104 to work toward control and eradication of severly destructive pests such as the brown marmorated stink bug, armyworm moths, and the Colorado potato beetle. Conversely, other National Programs have provided solutions for medical and veterinary entomology based on better application equipment, novel chemical formulation, and genomics. This sort of collaboration is based on recognizing mutually beneficial opportunities across disciplines or by sharing novel ideas for application to problems outside of a particular National Program. This process of scientific cross-fertilization is strengthened by the collocation of all NPLs within the Office of National Programs. NP 104 is in a particularly good position for this sort of interaction because its NPL also manages projects in Crop Production and Protection (projects in NP 304 and in NP 308, Methyl Bromide Alternatives), as well as serving as Acting Director of the Overseas Biological Control Laboratories.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

This report assembles some of the accomplishments of NP 104 from 2007 through 2011, a period that straddles the previous Action Plan for 2004-2008 and the current one for 2009-2013. Unfortunately, reporting on a period that does not match either Action Plan is necessary because of the need to review past accomplishments before beginning the process of developing a new Action Plan for the next 5-year cycle.

The NP 104 Accomplishment Report is a distillation of some of the most significant research accomplishments of the past 5 years achieved by scientists working in this National Program. By necessity, it is a 5-year snapshot that encompasses on-going research and the early benefits of that research. The content of this report is mostly derived from the annual reports of the NP 104 projects from the past 5 years. This report stresses the impacts of those accomplishments and, where relevant, cites key publications or Web links documenting those accomplishments.

This report was prepared for an external (to USDA) retrospective review of NP 104 to assess how well this National Program attained its projected goals, as outlined in its current Action Plan. Accordingly, the purpose of the retrospective review is not to judge the performance of individual NP 104 research projects, but to gauge the overall impact of the National Program. Consequently, the report does not attempt to catalogue all the individual accomplishments reported by the scientists assigned to NP 104's research projects.

The retrospective panel will be grading the accomplishments against the proposed actions outlined in the Problem Statements, which are more general than the anticipated products. An

individual accomplishment might be applicable to more than one problem and more than one set of anticipated products. An attempt has been made to match as many publications as possible with each accomplishment. However, with over 500 publications to consider, some of the matches may not be comprehensive. In a few cases, publications from 2012 were used because of a conspicuous accomplishment achieved in 2011 but not published until later. Appendix 1 includes a list of the current projects in NP 104, and Appendix 2 has a complete bibliography of peer-reviewed publications generated by the scientists in NP 104, organized by project. Appendix 3 contains a list of scientists in each laboratory, and Appendix 4 contains information regarding the relevance of NP 104 to the ARS Strategic Plan.

COMPONENT 1: Medical Entomology for the Public

Medical entomology may seem like an odd part of USDA research, but it has a distinguished tradition. The description of the first vector-borne pathogen, *Babesia bovis*, in cattle fever ticks was made by USDA scientist Theobald Smith in 1879. This discovery influenced the studies of Ronald Ross (malaria) and Walter Reed (yellow fever). USDA entomologists Leland O. Howard, Harrison G. Dyar, Jr., and Frederick Knab wrote the first comprehensive account of the mosquitoes of the Americas beginning in 1903 in response to the medical realization that mosquitoes were responsible for diseases such as yellow fever, filariasis, and malaria.

Compared with people in many other parts of the world, U.S. citizens suffer much less from diseases caused by arthropod-borne pathogens. Effective interventions based on sound medical entomology have had tremendous success in America against scourges such as malaria, filariasis, typhus, yellow fever, and dengue.

However, the West Nile virus epidemic that began in 1999 was a strong reminder that our country remains susceptible to these kinds of threats. Lyme disease, transmitted by certain species of ticks, is another persistent problem and appears to be getting worse over time. In addition, the discomfort caused by all kinds of arthropod bites drives the general public to actively seek relief on product shelves and from community governments. Therefore, improving the effectiveness, personal safety, and environmental safety of entomological interventions continues to be a priority outcome for research.

The research conducted within this component has implications for other aspects of the National Program. Nearly all of the work on medical entomology has relevance for the military (Component 2), although the emphasis in Component 1 is on domestic problems within the United States. Veterinary entomology (Component 3) also benefits from progress in medical entomology, sometimes directly from progress in controlling pests such as house flies and sometimes indirectly from the production of tools for control of a human pest (e.g., the Asian tiger mosquito), that has relevance for control of veterinary pests.

Within this component, most interactions with commercial companies involve control of the Asian tiger mosquito, the black-legged tick, and the house fly. That work has validated or repudiated product claims, refined the use of products, developed devices for commercial use, and provided guidance for better design of integrated pest management (IPM) programs. ARS has also been an active consultant to the Environmental Protection Agency (EPA) to provide advice on testing methods for repellents and public health pesticides. In addition, ARS participates in the Public Health Pesticide Consortium led by the Centers for Disease Control and Prevention and interacts with the World Health Organization's Pesticide Evaluation Scheme. Among the tangible products that have come out of this component are the 4-poster treatment bait station for control of ticks on deer and better traps for flies.

The accomplishments for Component 1 are aligned under the five Problem Statements noted in the Action Plan.

PROBLEM STATEMENT **1A:** *Priority species of mosquito (e.g., Aedes aegypti and Aedes albopictus, floodwater mosquitoes, Culex vectors of viruses, and Anopheles) are often controlled inefficiently because appropriate tools either do not exist or are not used in the most effective manner.*

The following locations have research projects addressing the issue identified under Problem Statement 1A:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas.
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.
- Biological Control of Pests Research Unit, National Biological Control Laboratory, Stoneville, Mississippi.
- European Biological Control Laboratory's satellite laboratory in Thessaloniki, Greece.

Problem Statement1A: Select Accomplishments

Interactions of mosquito receptor organs with repellents and attractants. Determining the mode of action of repellents and attractants has been a goal in medical entomology for at least the last 50 years. Only recently has the availability of genetic methods and representative dipteran genomes allowed comprehensive description of the proteins that interact with volatile and gustatory chemicals. Untangling the intricate associations between particular chemicals and mosquito receptor organs is of interest because of the importance of chemical cues used by mosquitoes as they complete various life-cycle requirements, such as host-recognition and oviposition site selection. A series of experiments by ARS researchers in Beltsville, Maryland, has added to our knowledge of how mosquito receptor organs work, with the implication that careful molecular design might create chemicals capable of disrupting reception much more efficiently than do current insect repellent active ingredients. The research has involved single-cell recording from antennae of *Aedes aegypti,* models of odorant-binding proteins expressed on frog oocyte membranes, and creation of transgenic mosquitoes that do not express the co-receptor protein commonly associated with binding proteins. This work was credited with discovering the first stereospecific odorant receptor ever found in mosquitoes. Most importantly, it has shown that active ingredients in repellents work differently from each other. Most of the active ingredients stimulate one kind of receptor and block another kind, apparently creating a false signal that makes it difficult for the mosquito to accomplish the final stages of host recognition. These results are an important step toward developing repellents and attractants that have much greater activity than do current compounds. By one estimate, using this approach might help to find compounds that are three orders of magnitude more active than current repellent active ingredients.

Bohbot, J.D., and Dickens, J.C. 2012. Odorant receptor modulation: Ternary paradigm for mode of action of insect repellents. Neuropharmacology 62.5: 2086-2095.

Grant, A.J., and Dickens, J.C. 2011. Functional characterization of the octenol receptor neuron on the maxillary palps of the yellow fever mosquito, *Aedes aegypti*. PLoS ONE 6(6): e21785. doi:10.1371/journal.pone.0021785

Bohbot, J.D., Fu, L., Le, T.N., Chauhan, K.R., Cantrell, C.L., and Dickens, J.C. 2011. Multiple activities of insect repellents on odorant receptors in mosquitoes. Medical and Veterinary Entomology dx.doi.org/10.1111/j.1365-2915.2011.00949.x.

Viruses for biocontrol of mosquitoes. Mosquito larvae are subject to many sources of mortality, including viral pathogens. An understanding of the conditions under which viral pathogens make a population-level change in mosquito numbers has interest in itself for risk assessment, but it may also lead to development of new larvicides. Research on viral pathogens of mosquitoes by ARS scientists in Gainesville, Florida, revealed that infection of larvae with at least some viruses is highly dependent on the ratio of calcium and magnesium ions in the water. The scientists discovered an unusual immune response by *Culex* mosquitoes to the mosquito-specific baculovirus, CuniNPV. Basically, the virus appears to shut down the ability of the mosquito to modify its cellular architecture, and the mosquito responds by overproduction of a chemical that regulates the process. *Culex* larvae were challenged with CuniNPV and the expression profile of the pro-apoptotic gene mx was measured. There was not a significant increase of mx expression before 8 hours post infection, but the level of mx expression continued to increase throughout the infection period and at 48 hours was 10 times higher than in the uninfected controls. The induction of mx did not result in apoptosis, but rather necrosis (degradation of cells), indicating that CuniNPV prevents apoptosis despite the very high level of mx expression. It is possible that CuniNPV utilizes an as-yet-unknown but powerful mechanism to block the apoptotic pathway downstream of mx activation. The physiology of the mosquito immune response could provide new targets for chemical control, and knowledge of viral-mosquito interaction will be important in designing effective viral larvicides.

Perera, O., Green, P.B., Stevens Jr., S.M., White, S., and Becnel, J.J. 2007. Proteins associated with *Culex nigripalpus* nucleopolyhedrovirus occluded virions. Journal of Virology 81.9: 4585-4590.

Integrated pest management for the Asian tiger mosquito. *Aedes albopictus* (the Asian tiger mosquito) is arguably the most problematic mosquito in the United States. It has behaved like the invasive species that it is, changing its biting habits from its native range to feed mostly on humans and occurring more commonly in urban situations. The species bites during the day, when people are most likely to encounter it, and it has spread through the southeastern quarter of the country, from Texas to Chicago and from New Jersey to Florida. The difficulty in controlling this mosquito is of real national concern because it is a powerful vector of the chikungunya virus, which caused millions of disease cases between 2004 and 2007 and spread from the Indian Ocean basin and Africa to Europe for the first time. ARS scientists in Gainesville, Florida, coordinated an areawide IPM project in 2008 to demonstrate how ARS technology could be assembled into an effective program. Working closely with Rutgers and Brandeis universities, the first 4 years of the program showed that a combination of careful surveillance using an attractant-based trap, targeted deployment of teams to eliminate larval sites, and ultra-low volume application of adulticides and herbicides could reduce the number of mosquitoes by about 70 percent. This program resulted in a 75 percent reduction in the number of weeks when mosquitoes were at an annoying level, thus enabling people to comfortably work outside for a greater part of the year. Just as importantly, economic surveys showed that people would be willing to pay approximately $20 per household per year for control of this mosquito, a figure in excess of current mosquito abatement budgets in the experimental areas.

Bartlett-Healy, K., Healy, S.P., and Hamilton, G.C. 2011. A model to predict evaporation rates in habitats used by container-dwelling mosquitoes. Journal of Medical Entomology 48.3: 712-716.

Doyle, M.A., Kline, D.L., Allan, S.A., and Kaufman, P.E. 2009. Efficacy of residual bifenthrin applied to landscape vegetation against Aedes albopictus. Journal of the American Mosquito Control Association 25.2: 179-183.

Obenauer, P.J., Kaufman, P.E., Allan, S.A., and Kline, D.L. 2009. Infusion-baited ovitraps to survey ovipositional height preferences of container-inhabiting mosquitoes in two Florida habitats. Journal of Medical Entomology 46.6: 1507-1513.

PROBLEM STATEMENT 1B**:** *Tick pests of humans (e.g., Ixodes scapularis and Ixodes pacificus [Lyme disease]; Dermacentor variabilis and andersoni [Rocky Mountain spotted fever]; Amblyomma americanum [Human Monocytic Ehrlichiosis]) are often controlled inefficiently because appropriate tools either do not exist or are not used in the most effective manner.*

The following locations have research projects addressing the issue identified under Problem Statement 1B:

- Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.
- Animal Parasitic Diseases Laboratory, Beltsville, Maryland.
- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.
- Yale University School of Medicine, New Haven, Connecticut (headquarters of a cooperative agreement).

Problem Statement 1B: Select Accomplishments

Lyme disease and black-legged ticks (Ixodes scapularis). Human cases of Lyme disease continue to increase, particularly in the northeastern United States and Wisconsin. ARS researchers in Kerrville, Texas, invented a system for treating deer to both remove attached ticks and prevent infestations of new ticks, thus greatly reducing the risk of Lyme disease. The technology, called the 4-poster deer treatment bait station, is now in commercial use on Gibson Island (Chesapeake Bay) and at the NASA Goddard Space Flight Center in Greenbelt, Maryland. Newer versions of the device are more practical in that they prevent disruption from raccoons, feral pigs, and other animals that steal the corn bait used in the 4-poster station. The refinement of these devices has been motivated not only by the need to control Lyme disease, but also by the need to treat deer in southern Texas for infestation by the cattle fever tick. The device is available commercially and has been used to reduce the risk of Lyme disease in New York, Connecticut, and Maryland. A modified device for control of the cattle fever tick is in wide use along the southern Texas border, where deer act as a reservoir for this important livestock pest.

Carroll, J.F., Pound, J.M., Miller, J.A., and Kramer, M. 2009. Sustained control of Gibson Island, Maryland, populations of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) by community-administered 4-Poster deer self-treatment bait stations. Vector-Borne and Zoonotic Diseases 9.4: 417-421.

Carroll, J.F., Hill, D.E., Allen, P.C., Young, K.W., Miramontes, E., Kramer, M., Pound, J.M., Miller, J.A., and George, J.E. 2009. The impact of 4-Poster deer self-treatment devices at three locations in Maryland. Vector-Borne and Zoonotic Diseases 9.4: 407-416.

Stafford III, K.C., Denicola, A.J., Pound, J.M., Miller, J.A., and George, J.E. 2009. Topical treatment of white-tailed deer with an acaricide for the control of *Ixodes scapularis* (Acari: Ixodidae) in a Connecticut Lyme borreliosis hyperendemic community. Vector-Borne and Zoonotic Diseases 9.4: 371-379.

Research on biology of Lyme disease spirochaete and the black-legged tick. For reasons that are not clear, the distribution of Lyme disease continues to expand. Any hope of preventing expansion of the disease requires an understanding of why it might be occurring in new places. ARS-funded research at Yale University on the biology of the Lyme disease spirochaete and the black-legged tick showed that Lyme disease spirochaetes occurred across the eastern part of the northern United States in a continuous distribution undisrupted by major alterations caused by human activity that began in the 18th and 19th centuries. Those alterations separated the spirochaete and its vector tick into smaller populations. Relict populations of the spirochaete are re-emerging as forest habitats are restored. During the course of this work, the researchers at Yale reported a new spirochaete pathogen that may be responsible for some of the observed disease. They also found that there is interaction between spirochaetes and *Babesia* parasites that modified pathogenicity. Climate change may extend the range of Lyme disease northwards, but it may also increase the occurrence of highly pathogenic spirochaetes. Finally, the researchers found that despite extensive attempts in colonized ticks, there was no sign of vertical transmission of the Lyme disease spirochaete. These findings have improved basic understanding of the most important vector-borne pathogen in the United States, with implications for design of IPM and medical programs.

Diuk-Wasser, M.A., Vourc'h, G., Cislo, P., Hoen, A.G., Melton, F., Hamer, S.A., Rowland, M., Cortinas, R., Hickling, G.J., Tsao, J.I., Barbour, A.G., Kitron, U., Piesman, J., and Fish, D. 2010. Field and climate-based model for predicting the density of host-seeking nymphal *Ixodes scapularis*, an important vector of tick-borne disease agents in the eastern United States. Global Ecology and Biogeography 19.4: 504-514.

Platonov, A.E., Karan, L.S., Kolyasnikova, N.M., Makhneva, N.A., Toporkova, M.G., Maleev, V.V., Fish, D., and Krause, P.J. 2011. Humans infected with relapsing fever Spirochete *Borrelia miyamotoi*, Russia. Emerging Infectious Diseases 17(10): 1816-1823.

Margos, G., Vollmer, S.A., Ogden, N.H., and Fish, D. 2011. Population genetics, taxonomy, phylogeny and evolution of *Borrelia burgdorferi* sensu lato. Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases 11(7): 1545-1563.

Repellents against ticks. Repellents are commonly used to prevent tick bites, but some ticks are not as easily repelled as others. ARS scientists in Beltsville, Maryland, designed systematic tests of repellents to measure the ability of a chemical to interrupt movement of a tick. These tests indicated that the best active ingredients were different for the Lyme disease tick than for the

lone star tick (*Amblyomma americanum*). Duration of tick repellency was generally less than for mosquitoes. The scientists demonstrated that some new compounds, both synthetic and of natural origin, were more effective than currently registered products. EPA signed a memorandum of understanding with ARS in 2010 under which ARS scientists offered expert opinions on new rules for standardized testing of repellents against ticks. This work provided EPA with a standardized testing procedure it could require from industry as well as a method for prioritizing the effectiveness of a series of active ingredients.

Carroll, J.F. and Kramer, M.H. 2012. Responses of Lone Star Tick (Acari: Ixodidae) nymphs to the repellent deet applied in acetone and ethanol solutions in in vitro bioassays. Journal of Entomological Science 47:193-196.

Carroll, J.F., Benante, J.P., Kramer, M.H., Lohmeyer, K.H., and Lawrence, K. 2010. Formulations of Deet, Picaridin and IR3535 applied to skin repel nymphs of the Lone Star Tick (Acari: Ixodidae) for 12 hours. Journal of Medical Entomology 47(4):699-704.

Carroll, J.F., Klun, J.A., and Kramer, M. 2008. Similarity in responses of laboratory-reared and field-collected lone star tick (Acari:Ixodidae) nymphs to repellents. Journal of Entomological Science 43(4):426-430.

PROBLEM STATEMENT 1C**:** *Flies, as mechanical vectors of pathogens to humans and food, are generally controlled poorly, resulting in standing populations capable of inoculating food and wounds with pathogens.*

The following location has research projects addressing the issue identified under Problem Statement1C:

- Mosquito and Fly Research Unit, Center for Medical Agricultural and Veterinary Entomology, Gainesville, Florida.

Problem Statement 1C: Select Accomplishments

Attractants for house fly control. Flies are very difficult to control in an agricultural setting because there are so many larval sources in environments where animals are raised. A number of methods are used to reduce the number of adult flies, including attraction to traps and insecticidal baits. ARS researchers in Gainesville, Florida, conducted testing in large outdoor cages with a seven-component blend based on black strap molasses to attract house flies. The blend was found to be as attractive as natural molasses lures. Dose-response studies demonstrated that although flies can detect a 3 percent dilution of the attractant as currently formulated, optimal results are obtained with a 10 percent dilution. The blend was found to be much more attractive to previtellogenic flies than to older, gravid flies. A variety of natural sugar products were also tested for relative attractiveness to identify additional sources of fly attractants. Molasses was found to be substantially more attractive than honey, maple syrup, barley malt syrup, or jaggery (date palm sugar). A patent for the attractant was issued in November 2011. These results have been used to produce improved fly baits.

Quinn, B.P., Bernier, U.R., and Booth, M.M. 2007. Identification of compounds from etonia rosemary (Conradina etonia). Journal of Chromatography 1160:306-310

Quinn, B.P., Bernier, U.R., Geden, C.J., Hogsette Jr, J.A., and Carlson, D.A. 2007. Analysis of extracted and volatile components in blackstrap molasses feed as candidate house fly attractants. Journal of Chromatography 1139(2):279-284

Patented device for house fly control. Adult house flies are an almost inevitable part of livestock and poultry production because of the difficulty of eliminating all larval sources; therefore, adult fly control can be very important for reducing the presence of flies. ARS scientists in Gainesville, Florida, developed a lightweight inexpensive trap, named the Flybrella, that can be suspended like an upside down umbrella in locations where house flies rest. The trap consists of a solid, cylindrical backbone to which a perforated transparent tube with a commercial sugar/toxicant strip affixed inside is attached. Centered directly beneath the tube and also attached to the backbone is a 10-cm-diameter cone. House flies readily enter the tube, feed on the fast-acting toxicant, and fall directly down the tube to an inverted opaque plastic cone where they are concealed. The cone may be emptied easily through a capped opening in the bottom. In paired indoor tests, the efficacy of the commercial sugar/toxicant strip was increased significantly by the individual addition of several other attractant materials. Variants of the original design were tested, including a more efficient design featuring two concentric tubes with offset perforations. Design of the trap allows the toxicant strip to be easily removed and/or replaced when desired. This design has led to better commercial traps based on similar requirements.

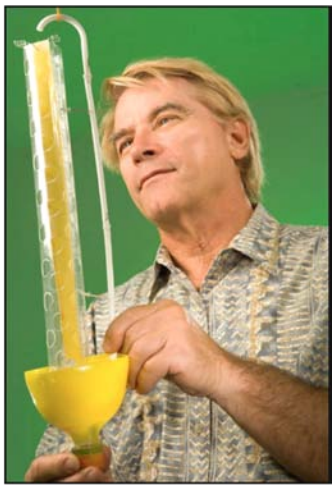

FIGURE 1: ARS chemist David Carlson examines a Flybrella trap, which attracts flies to the bait strip inside the clear cylinder. The flies feed, die, and drop into the yellow umbrella.

Carlson, D.A. and Hogsette Jr, J.A. 2007. Flybrella: a device to attract and kill house flies. Journal of Economic Entomology 100:483-487.

Advances in fly biological control. Biological control of flies is an appealing strategy in that it does not use chemicals, it may be less susceptible to resistance than chemical control, and it is completely safe. Although biological control agents are used routinely in poultry operations, the level of control is inconsistent. The development of different kinds of biological control agents may improve the potential for this strategy to be widely used. The parasitic wasp, *Trichopria nigra*, was originally obtained from stable fly pupae in Russia and Kazakhstan by an ARS collaborator. In the absence of experience with any host material, this species is strongly attracted to pupae of the house fly, stable fly, horn fly, black dump fly, and flesh flies. Behavioral studies by ARS scientists at Gainesville, Florida, have demonstrated that 24 hours of conditioning of young parasitoids on one species strongly influences the attractiveness of that species in subsequent exposures. The results may facilitate mass production of this species and lead to ways to improve the effectiveness of released biocontrol agents against intended target species. The use of this potential biological control agent may be improved greatly by rearing it in the presence of the intended target fly species.

Handling biocontrol agents was made easier by a discovery by ARS scientists in Gainesville, Florida. They found that disabled house fly pupae could be used for rearing parasitoids. By using heat shock of house fly puparia, no house flies could emerge, but the puparia were still

capable of supporting successful development of the common commercial parasitoids, *Spalangia cameroni* and *Muscidifurax raptor*. Freezing and gamma irradiation also proved useful for this purpose. This method may be adapted to commercial mass rearing to reduce costs associated with continuous maintenance of house fly colonies.

Geden, C.J. 2007. Development of spalangia cameroni and muscidifurax raptor on live house fly pupae and pupae killed by heat shock, irradiation and cold. Environmental Entomology 36(1):34-39.

Geden, C.J. and Moon, R.D. 2009. Host ranges of gregarious muscoid fly parasitoids: *Muscidifurax raptorellus* (Kogan and Legner) (Hymenoptera:Pteromalidae), *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae), and Trichopria (Hymenoptera: Diapriidae). Environmental Entomology 38(3):700-707.

Kaufman, P.E., and Geden, C.J. 2009. Development of *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) on live and freeze-killed house fly (Diptera: Muscidae) pupae. Florida Entomologist 92(3)492-496.

PROBLEM STATEMENT 1D**:** *Bed bugs have become much more abundant in the United States during the last five years, presenting the need for better surveillance and control technology.*

The following location has research projects addressing the issue identified under Problem Statement 1D:

- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Problem Statement 1D: Select Accomplishments

Chemical ecology and control of bed bugs. Although the two predominant defensive secretions (alarm pheromones) of the bed bug have been known since the 1960s, little information existed on any additional compounds that might play a role in chemical communication among bed bugs. ARS scientists in Beltsville, Maryland, characterized the hydrocarbons associated with the bed bug cuticle and also identified two new defensive secretions from the dorsal abdominal glands of bed bug nymphs. The scientists demonstrated that cuticular extracts were involved in the bed bug behavior of aggregation. ARS personnel maintain several laboratory and field-collected strains of bed bugs, including strains that are sensitive to pyrethroid insecticides and strains that are pyrethroid resistant. In an effort to discover new, efficacious compounds to control bed bugs, these strains have been used in toxicity assays of existing insecticides, compounds developed by ARS, and compounds supplied by industry. Although the vast majority of compounds tested proved ineffective, two pyrethroid compounds synthesized by an ARS chemist in Beltsville, Maryland, were toxic to a bed bug strain that was highly resistant to a commonly used pyrethroid insecticide, deltamethrin. In a recently completed laboratory study, ARS scientists in Beltsville demonstrated that male bed bugs could be used exclusively in testing protocols, thereby preserving females for colony maintenance and expansion, and they showed that there is little reason to assess toxicity of chemicals beyond 1 week, even when bugs are exposed for only 24 hours. ARS participates in the National Bed Bug Task Force, which

includes seven Federal agencies working on a national strategy to mitigate the impact of bed bugs. In addition, ARS participates on an EPA Scientific Advisory Panel convened to review efficacy and harmonize the laboratory testing of bed bug pesticides.

Feldlaufer, M.F. and Blomquist, G. 2011. Cuticular hydrocarbons from the bed bug *Cimex lectularius* L.. Biochemical Systematics and Ecology 39:283-285.

Feldlaufer, M.F., Domingue, M.J., Chauhan, K.R., and Aldrich, J.R. 2010. 4-Oxo-Aldehydes from the dorsal abdominal glands of the bed bug (hemiptera: cimicidae). Journal of Medical Entomology 47(2):140-143.

Domingue, M.J., Kramer, M.H., and Feldlaufer, M.F. 2010. Sexual dimorphism of bed bug (Cimex lectularis) attraction and aggregation responses to cuticular extracts from nymph exuviae. Physiological Entomology 35(3):203-213.

PROBLEM STATEMENT 1E**:** *Invasive vectors of human pathogens should be detected early, stopped before they can establish themselves, or eradicated if they become established.*

The following location has research projects addressing the issue identified under Problem Statement 1E:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.

Problem Statement 1E: Select Accomplishments

Risk assessment and surveillance of vectors of Rift Valley fever virus. To prevent potential introduction of the Rift Valley fever virus into the United States, detection and response to outbreaks in natural settings in Africa and the Middle East are crucial. In 2007, ARS scientists participated in the accurate forecast of a Rift Valley fever outbreak in Kenya. Researchers discovered a method using global and local climate and vegetation development information to forecast this disease well before it occurs. In late 2006, an early warning of an impending Rift Valley fever outbreak was issued to national and international agricultural and public health officials 3 months before a large outbreak occurred in four countries in the Horn of Africa. This outbreak affected thousands of people and hundreds of thousands of domestic animals, dramatically impacting the economy of these regions. This alert resulted in increased national and international surveillance and dramatically enhanced the Rift Valley fever control response for the first time in history, reducing the impact of the disease and protecting other countries from becoming affected by the disease.

The same technique has been used to examine the potential for Rift Valley fever transmission in the United States by examining remote sensing data on vegetation index and temperature. GIS models of climate-population and habitat-population associations to predict temporal and spatial distribution and abundance of medically important mosquito species were developed, taking into account those U.S. species shown to be competent vectors. A spatially explicit bionomics database is being expanded for quick reference and triage should a mosquito-borne disease emergency strike one or several U.S. regions simultaneously. Integrating these products with the African remote-sensing Rift Valley fever virus prediction model will lead to a virus early-

warning GIS for the United States that will target key areas such as ports for heightened disease surveillance, prevention, and response efforts, thus potentially reducing the severity of future exotic mosquito-borne disease outbreaks in this country.

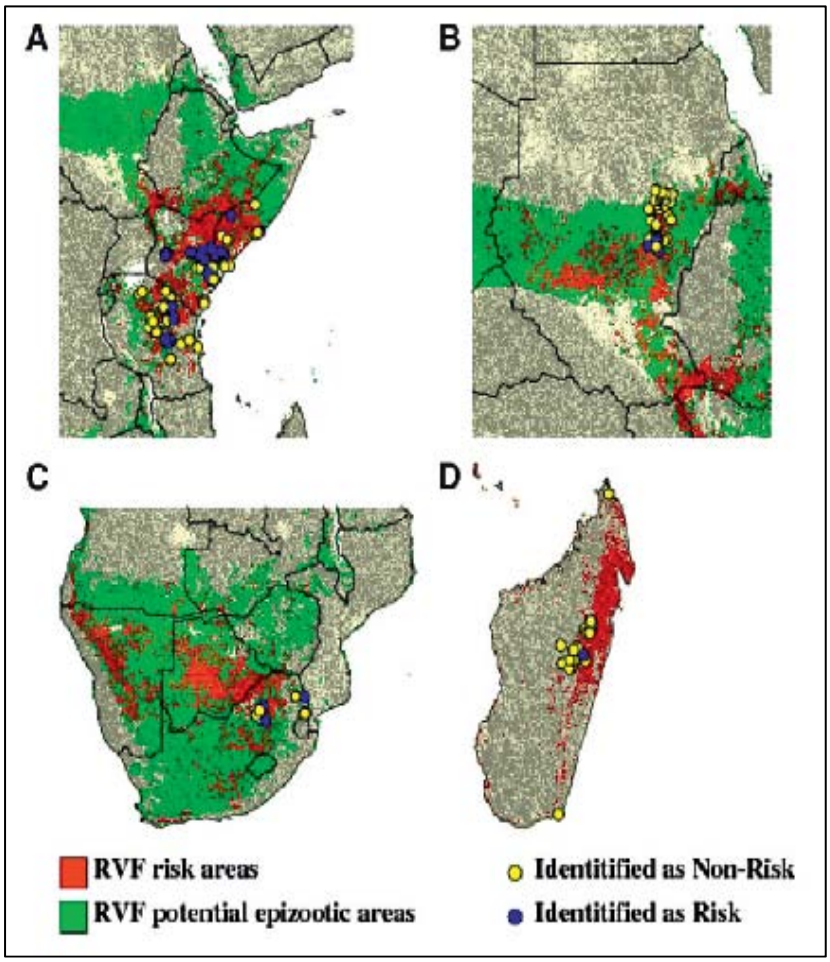

FIGURE 2: Summary Rift Valley fever risk maps are shown for (A) Eastern Africa: 2006-2007; (B) Sudan: 2007; (C) Southern Africa: 2007-2008; (D) Madagascar: 2007-2008. Areas shown in green represent potential epizootic areas for the period stated, while red represent pixels mapped by the prediction system to be at risk for fever activity. Blue dots indicate human cases identified to be in risk areas, while yellow dots represent human cases in areas not mapped to be at risk.

ARS participated in the establishment of a Rift Valley Fever Virus Working Group consisting of multiple government agencies. Although U.S. agencies and universities are actively creating response/surveillance plans for Rift Valley fever virus and researching critical technological advances such as diagnostics and vaccines, improvements are needed in communication and integrated response efforts should the virus unexpectedly arrive in the United States. The Working Group continues to have a measurable positive impact on Rift Valley fever research directions and outcomes and sets a trajectory that will significantly strengthen and protect the United States against the possible arrival of the virus as well as other mosquito-borne viruses currently or potentially impacting the U.S. economy and public and veterinary health.

Anyamba, A., Linthicum, K., Small, J., Britch, S.C., Pak, E., De La Rocque, S., Formenty, P., Hightower, A.W., Breiman, R., Chretien, J., Tucker, C.J., Schnabel, D., Sang, R., Haagsma, K., Latham, M., Lewandowski, H.B., Osman Magdi, S., Mohamed, M., Nguku, P.M., Reynes, J., and Swanepoel, R. 2010. Prediction, Assessment of the Rift Valley fever activity in east and southern Africa 2006 - 2008 and possible vector control strategies. American Journal of Tropical Medicine and Hygiene 83(2):43-51.

Turell, M.J., Linthicum, K., Patrican, L.A., Davies, F.G., Kairo, A., and Bailey, C.L. 2008. Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. Journal of Medical Entomology 45(1):102-108.

Britch, S.C., Linthicum, K., Anyamba, A., Tucker, C.J., Pak, E., Maloney, Jr., F.A., Cobb, K., Stanwix, E., Humphries, J., Spring, A., Pagac, B., and Miller, M. 2008. Satellite vegetation index data as a tool to forecast population dynamics of medically important mosquitoes at military installations in the continental United States. Military Medicine 173(7):677-683.

Climate change and vector-borne disease. Because the activity and distribution of arthropods are closely linked to temperature and moisture, it is broadly assumed that one of the effects of global climate change will be a redistribution of vector-borne pathogens. ARS is represented on the Interagency Working Group on Climate Change and Health, assembling information on the subject in a form that could be influential among policy makers and scientists not involved in entomology. The Group published a coordinated report that, with respect to vector-borne diseases, concluded that climate change could result in extension of range of some pathogens because of redistribution of vectors and possibly accelerated development of pathogens in vectors. The report indicated that ecological disruptions unrelated to vectors might increase the likelihood of successful establishment of invasive species, including vectors. This effort documented that climate change is likely to influence public health and agriculture with respect to vector-borne pathogens.

Portier, C.J., Thigpen Tart, K., Carter, S.R., Dilworth, C.H., Grambsch, A.E., Gohlke, J., Hess, J., Howard, S.N., Luber, G., Lutz, J.T., Maslak, T., Prudent, N., Radtke, M., Rosenthal, J.P., Rowles, T., Sandifer, P.A., Scheraga, J., Schramm, P.J., Strickman, D., Trtanj, J.M., and Whung, P-Y. 2010. A Human Health Perspective On Climate Change: A Report Outlining the Research Needs on the Human Health Effects of Climate Change. Research Triangle Park, NC: Environmental Health Perspectives/National Institute of Environmental Health Sciences doi:10.1289/ehp.1002272. www.niehs.nih.gov/climatereportInteragency

Changes in epidemiological patterns of West Nile virus transmission. Longitudinal studies conducted by ARS researchers in Thessaloniki, Greece, using mosquito trapping and sentinel chicken flocks in northern Greece have demonstrated the successful overwintering of a highly pathogenic strain of West Nile virus. This was the first observation that this virus had overwintered in Europe and may indicate that global climate change has extended the endemic range of the virus northward. Combined with possible new mosquito associations (*Culex modestus*), the situation also raises concerns about whether introduction of this mosquito species to the United States or other countries would further exacerbate the West Nile virus situation outside of Greece. These results showed that risk assessment should include monitoring of potentially invasive species that could have a large impact on transmission of pathogens outside of their native ranges.

Chaskopoulou, A., Dovas, C. I., Chaintoutis, S. C., Bouzalas, I., Ara, G., and Papanastassopoulou, M. 2011. Evidence of enzootic circulation of West Nile virus (Nea Santa-Greece-2010, lineage 2), Greece, May to July 2011. Euro Surveill 16, 19933.

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COMPONENT 2: Military Medical Entomology

The U.S. military is one of the few armed forces in the world that is able to deploy large numbers of forces and keep them healthy in challenging environments. However, one of the major health threats that even a well-equipped military can experience comes from vector-borne pathogens, particularly those that cause diarrheal disease, malaria, dengue, and leishmaniasis.

In 1942, the U.S. military enlisted the help of USDA's Orlando Laboratory, the direct antecedent of the present-day ARS Gainesville, Florida, laboratory, to develop methods for controlling plague, malaria, scrub typhus, and epidemic typhus. The laboratory's successful efforts included effective clothing repellents, topical repellents, insecticides, and the aerosol can. Those accomplishments can be directly credited for the advantage enjoyed by Allied troops in North Africa, southern Europe, and the Pacific Theater during World War II. The association with the military has continued, stimulating the invention of DEET and *para*-menthane diol insect repellents, the development of ultra-low volume insecticide application for mosquitoes, the use of permethrin as a clothing impregnant, and the invention of molecular pesticides.

Since 2004, the Department of Defense (DoD) has provided annual funding to ARS as part of the Deployed Warfighter Protection Program, which stimulated the inclusion of this component in NP 104. This history of achievement for the protection of public health from biting pests was a direct result of the entomological expertise developed mainly for agriculture. In recent years, the ARS-U.S. military collaboration has focused on preventive medicine and a capability for entomological intervention, including development of new tools for the control portion of IPM. The research specifically targets the development of new pesticides, new personal protection systems (e.g., bed nets, repellents), and new application equipment.

As mentioned above, medical entomology for the public has considerable relevance for military entomology, especially in the production of new tools that can be used during a deployment and domestically. Veterinary entomology also produces tools that can be used by the military overseas, either for control of potential zoonotic vectors or as spin-off inventions that can be adapted to protecting humans. Although outside of the mission of the Deployed Warfighter Protection Program, accomplishments in Components 4 and 5 (termites and ants) also address problems commonly experienced by the military both in the United States and abroad.

This component has had the most interaction with commercial companies, aggressively negotiating relationships and finding partners for particular products. The development of a new insecticide generally takes many years; therefore, it is not surprising that none of the active ingredients invented by ARS have come to the marketplace, but progress is being made. More immediate results have been achieved with devices such as traps, expansion of labels of existing products, and refinement of application techniques. ARS has also played a significant role in discussion with EPA on registration of pesticides for use against vectors that do not occur in the United States and on registration of RNA interference (RNAi)[1] insecticides.

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[1] RNAi—the natural process usually initiated by a double-stranded RNA with a sequence mirroring part of a transfer RNA and resulting in destruction of the transfer RNA, inhibiting production of a specific protein.

The accomplishments for this Component are aligned under the three Problem Statements as noted in the Action Plan.

PROBLEM STATEMENT 2A**:** *Toxicants currently available for controlling arthropod vectors are not adequate to protect deployed military personnel.*

The following locations have research projects addressing the issue identified under Problem Statement 2A:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.
- Natural Products Utilization Research Unit, Oxford, Mississippi.
- Biological Control of Pests Research Unit, National Biological Control Laboratory, Stoneville, Mississippi.
- European Biological Control Laboratory's satellite laboratory in Thessaloniki, Greece.

Problem Statement 2a: Select Accomplishments

Improving filth fly traps. Filth flies, particularly the house fly, are attracted to the water sources and fecal odors of American military encampments. Moderate and large fly populations increase the risk of diarrheal disease, the primary cause of lost duty time in deployed American military personnel, by amplifying pathogenic bacteria in their guts and by mechanically transmitting the bacteria from feces. ARS scientists in Gainesville, Florida, compared common types of commercial fly traps and found a wide variation in efficacy. The scientists showed that the trap currently used by the military was not very efficient. ARS worked with the Armed Forces Pest Management Board to add a more efficient fly trap to the logistics system, which makes the trap available to deployed military personnel. The ARS scientists also developed a field-expedient fly trap made from a water bottle, demonstrating that simply painting the top of the trap black increased catches by six-fold. Two new synthetic attractant blends developed by ARS (one based on analysis of chemical attraction and the other based on Chinese natural products) could replace foul-smelling and messy baits currently in use in fly traps. Better, more practical tools for fly control could reduce the rate of non-battle disease from rates as high as 20 percent per week down to 4 percent per week. The impact of the availability of healthy military personnel acts as a force multiplier, reducing the number of personnel who must be deployed to accomplish the mission.

Geden, C.J., Szumlas, D.E., and Walker, T.W. 2009. Evaluation of commercial and field-expedient baited traps for house flies, *Musca domestica* L. (Diptera: Muscidae). Journal of Vector Ecology 34(1):99-103.

New chemicals for mosquito and vector control. New active ingredients for vector control are seldom developed. As existing active ingredients are eliminated by regulatory concerns and development of resistance, a gap develops in the ability to control mosquitoes. ARS scientists in Gainesville, Florida; Oxford, Mississippi; Beltsville, Maryland; and Kerrville, Texas, performed extensive discovery and development work to create entirely new candidate chemicals for

protection of military personnel and for public health use. Some of these compounds may prove useful for veterinary application as well. The ARS laboratories share expertise in a coordinated strategy similar to industrial scale efforts. The strategies for discovery have been varied, ranging from medium through-put screening to intuitive discovery of organic molecules to highly targeted disruption of physiological processes. Systematic examination of piperidine compounds with modification of some molecular side groups resulted in development of one new molecule that is highly toxic to mosquitoes. A new system for high-throughput screening of trial compounds using first instars of mosquito larvae was developed and used to screen over 2,000 compounds, including a few that have promising commercial potential. Examination of natural products derived from fungi resulted in development of a new class of toxicants derived from chromenes.

An ARS collaborator at the University of Florida has specialized in development of highly specific acetylcholine esterase inhibitors that may reintroduce this kind of pesticide, but in a form that is much safer than legacy organophosphates and carbamates. The collaborator has been able to take advantage of ARS research on basic acetylcholinesterase structure of other pests, extending the application of these efforts. Another successful research direction has been the invention of a range of pyrethroids that vary in volatility, some of which avoid the use of halogens. These compounds not only offer some intriguing possibilities for new uses, but they also overcome resistance of some pests by delivering an overwhelming dose to the target site. ARS is actively engaged in development efforts as well, working with its own Office of Technology Transfer, EPA, the IR-4 program, and industry (through Cooperative Research and Development Agreements).

ARS scientists in Gainesville, Florida, invented one of the first RNAi-based insecticides, showing that double-stranded RNA (dsRNA) inhibiting an apoptosis-regulating protein could kill mosquitoes following injection, feeding, or transcuticular transport. ARS received a patent for this product in December 2011. This technology has been expanded greatly by commercial efforts, and ARS is partnering with industry to develop products that would work against ants, mosquitoes, and sand flies. RNAi products may be much easier to register, much safer in the environment, and able to avoid resistance problems.

Kamalakannan, S., Murugan, K., and Barnard, D.R. 2011. Toxicity of *Acalypha Indica* (Euphorbiaceae) and *Achyranthes aspera* (Amaranthaceae) leaf extracts to *Aedes aegypti* (Diptera: Culicidae). Journal of Asia-Pacific Entomology 14.1: 41-45.

Meepagala, K.M., Schrader, K., Burandt, C., Wedge, D.E., and Duke, S.O. 2010. New class of algicidal compounds and fungicidal activities derived from a chromene amide of *Amyris texana*. Journal of Agricultural and Food Chemistry 17:(58)9476-9482.

Pridgeon, J.W., Zhao, L., Becnel, J.J., Strickman, D.A., Clark, G.G., and Linthicum, K. 2008. Topically applied AaeIAP1 double-stranded RNA kills female adults of *Aedes aegypti*. Journal of Medical Entomology 45(3):414-420.

Pridgeon, J.W., Zhao, L., Becnel, J.J., Clark, G.G., and Linthicum, K.J. 2008. Developmental and environmental regulation of AaeIAP1 transcript in *Aedes aegypti*. Journal of Medical Entomology 45(6):1071-1079.

Using existing products more effectively to protect military personnel. Protection of deployed military from mosquitoes and flies that transmit disease is a critical component of successful military operations because U.S. military personnel are generally susceptible to the pathogens and are exposed to bites in the field. ARS researchers in Gainesville, Florida, carried out field trials of two insecticides (bifenthrin and lambda-cyhalothrin) as barrier treatments (applied to vegetation or to military materials between the source of biting arthropods and the location to be protected) in desert environments (the Coachella Valley in California; western Kenya; Iraq; and Afghanistan) and in a humid sub-tropical environment in Florida. Results from vegetation treatments indicated a significant reduction in mosquitoes in field counts and lab assays for up to a month. Results from material treatments indicated significant reduction of mosquitoes or sand flies in field counts and lab assays for up to 18 months.

Another way to reduce the number of vectors is application of aerosols of insecticide outdoors. Trials in a desert environment showed that thermal fog (produced by machines that burn a mixture of insecticide and oil to create a dense insecticide-laden smoke) was superior to ultra-low volume application, which produces very small droplets in a mist that is made mechanically, and that malathion, an organophosphate, was better than sumithrin, a pyrethroid. The results suggest that, as an enhancement to the current DoD pest management system (such as use of DEET and permethrin treatment of uniforms), barrier treatments may be successful in providing protection from vector-borne diseases for deployed troops in desert habitats by significantly reducing densities of mosquitoes or sand flies reaching individual personnel in protected areas. Thermal fogs of malathion were shown to be better than the usual treatment involving ultra-low volume pyrethroids. Helicopter aerial application of ultra-low volume in Greece, using a European-registered aqueous deltamethrin product, showed greater than 75 percent kill of West Nile virus vectors in large plots with no adverse effects on bees or other insects. The technology included highly accurate targeting methods that modeled weather effects as the aerosol was applied, making it possible to make applications precisely while flying at 100 miles per hour.

Chaskopoulou, A., Latham, M.D., Pereira, R.M., Connelly, R., Bonds, J.A., and Koehler, P.G. 2011. Efficacy of aerial ultra-low volume applications of two novel water-based formulations of unsynergized pyrethroids against riceland mosquitoes In Greece. Journal of the American Mosquito Control Association 27(4), 414-422.

Registration of existing chemicals for protection of military personnel. The IR-4 Project, based at Rutgers University, is funded by the USDA National Institute of Food and Agriculture (NIFA) and ARS to facilitate registration of pesticides for minor uses that would not otherwise justify the investment for private industry. Scientists have successfully provided specialty crops with the range of toxicants required to achieve efficient production, gaining the trust of both EPA and industry. In 2008, ARS and the Armed Forces Pest Management Board developed funding and an agreement with IR-4 to work on public health pesticides, with the goals of facilitating registration of new compounds, adding military use indications to existing products, and examining the suitability of new chemistries. During this reporting period, IR-4 worked with ARS, industry, and other laboratories to get preliminary registration of etofenprox as an ultra-low volume application insecticide, the first new active ingredient for this use in 20 years. The program has also begun discussions with EPA on registration issues likely to surround dsRNA, organized experimental use permits for areawide application of pyriproxyfen for mosquito

control, and helped obtain the first military use indication (for a rodent feed-through product that kills associated sand flies) in over 30 years. (http://ir4.rutgers.edu/publichealth.html)

PROBLEM STATEMENT 2B: *Personal protection products against arthropod vectors are not capable of providing optimal protection for military personnel.*

The following locations have research projects addressing the issue identified under Problem Statement 2B:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.
- Natural Products Utilization Research Unit, Oxford, Mississippi.

Problem Statement 2B: Select Accomplishments

Research toward understanding how repellents work. Current repellents and attractants require high concentrations to achieve their desired effects on vector behavior. A more precise understanding of the physiology of odor reception could lead to the discovery of compounds that affect mosquitoes at much lower concentrations. In research to better understand how mosquitoes detect attractant odors, ARS scientists in Beltsville, Maryland, generated transgenic *Aedes aegypti* mosquitoes in which the expression and activities of specific olfactory (odor-detecting) genes are reduced. These genes include AeOrco, an obligatory partner of most odor receptors, and AeOR8, an odor receptor responsible for the detection of an odor released by animal hosts. The resulting transgenic mosquitoes were characterized to more fully elucidate the functions of these genes in mosquitoes. Two additional genes, SNMP1 and SNMP2, have broad roles in smell in most insects, including mosquitoes; these genes have been deleted from the fruit fly (a close relative of mosquitoes), and the resulting flies are being investigated behaviorally and physiologically to elucidate the functions of these genes. The regulatory regions of SNMP1 and SNMP2 genes have been dissected in transgenic flies to identify molecular mechanisms that determine classes of chemosensory organs in order to develop strategies for disrupting broad chemosensory capabilities. Identification of the precise role of particular genes in host attraction will lead to new designs for molecules that disrupt mosquito behavior.

Vogt, R.G., Miller, N.E., Litvack, R., Fandino, R.A., Sparks, J., Staples, J., Friedman, R. and Dickens, J.C. 2009. The insect SNMP gene family. Insect Biochemistry and Molecular Biology 39(7):448-456.

Influences on responses to repellents. Viral infection of mosquitoes may be important in modifying mosquitoes' responses to repellents. If viral infection causes tolerance to repellents, then the use of repellents might actually tend to select for those mosquitoes in the area that are infected. Venezuelan equine encephalitis virus (VEEV)-infected *Aedes taeniorhynchus* mosquitoes and their "sham-infected" siblings (i.e., inoculated with VEEV or diluent, respectively) were compared by ARS scientists in Gainesville, Florida, in cooperation with the U.S. Army Medical Research Institute on Infectious Diseases in Fort Detrick, Maryland. Infected mosquitoes bit a repellent-treated (DEET) hamster before uninfected mosquitoes bit the hamster. In other experiments, VEEV-infected mosquitoes were not stopped by masking-agent

chemicals, whereas uninfected mosquitoes did not bite a hamster protected by those agents. Work with dengue failed to show any effect on repellent response, and experiments are being undertaken using Rift Valley fever virus. For some virus-mosquito systems, there is a danger that use of repellents may actually select for bites from infected mosquitoes. Although it is too early to make recommendations, this work suggests that additional research is warranted to understand how virus infection modifies behavior to be able to best use repellents for disease prevention.

Frances, S.P., Sithiprasasna, R., and Linthicum, K. 2011. Laboratory evaluation of the response of *Aedes aegypti* and *Aedes albopictus* uninfected and infected with dengue virus to DEET. Journal of Medical Entomology 48(2):334-336.

Allan, S.A., Bernier, U.R. and Kline, D.L. 2010. Laboratory evaluation of human-associated odors on attraction of Culex spp. (Diptera: Culicidae). Journal of Vector Ecology 35:1-7.

Bernier, U.R., Kline, D.L., Allan, S.A., and Barnard, D.R. 2007. Laboratory comparison of *aedes aegypti (*l.) attraction to human odors and to synthetic human odor compounds and blends. American Mosquito Control Association 23(3) 288-293.

Evaluation and innovation of treated uniforms. The U.S. military has used permethrin treatment of uniforms since 1990 to prevent bites of mosquitoes through clothing and tick and chigger bites. New uniforms use different textiles, some of which are not as suitable for permethrin treatment, and the military is using more factory-treated uniforms instead of treating uniforms with dips or sprays in the field. Factory treatment creates a challenge for quality control. ARS scientists in Gainesville, Florida, developed quantitative methods for testing the quality of factory treatment and also for trial treatments that overcome the lack of absorption of permethrin by some fibers. In addition, ARS scientists in Beltsville, Maryland, are developing an entirely new system for protection. This system involves volatilization of spatial repellents from several points of the uniform, protecting military personnel from bites through the uniform and on adjacent exposed skin. This technique involves co-distillation of two or more active ingredients, achieving a greater concentration of the chemicals in the air layer close to a person. An industrial partner is making trial products using this principle, which are then tested by ARS.

Hoel, D., Pridgeon, J.W., Bernier, U.R., Chauhan, K., Meepagala, K., and Cantrell, C. 2010. Departments of Defense and Agriculture team up to develop new insecticides for mosquito control. Wing Beats 21(1):29-34.

Linthicum, K. J., Allan, S., Barnard, D., Becnel, J., Bernier, U. R., Britch, S., Clark, G., and Zhao, L. 2007. Mosquito and fly control research by the USDA-ARS Center for Medical, Agriculture and Veterinary Entomology (CMAVE) in the Deployed War-Fighter Protection (DWFP) program. Proceedings and Papers of the 75th Annual Conference of the Mosquito and Vector Control Association of California, February 4-7, 2007. http://digitalcommons.unl.edu/usdaarsfacpub/1033

PROBLEM STATEMENT 2C: *Application techniques and equipment for dispersal of pesticides are not adequate to protect deployed military personnel from pathogens transmitted by arthropods. Identification of an effective active ingredient is only part of what is needed for a good insecticidal treatment. Formulation of the active ingredient imparts characteristics necessary for a particular use. For example, good spreading characteristics and long residual*

would be desirable for a barrier treatment applied to vegetation. The equipment dispensing the pesticide is also important, since it controls many factors that affect final distribution of the toxicant.

The following locations have research projects addressing the issues identified under Problem Statement 2C:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Areawide Pest Management Research Unit, College Station, Texas.

Problem Statement 2C: Select Accomplishments

Development of International Guidelines for Insecticides and Repellents. At the invitation of the Director of the World Health Organization (WHO) Pesticide Evaluation Scheme, ARS scientists played leadership roles with other international experts in the development of WHO Guidelines for:

(1) Efficacy Testing of Mosquito Repellents for Human Skin. (http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.4_eng.pdf);
(2) Efficacy Testing of Insecticides for Indoor and Outdoor, Ground-applied Space Spray Applications. (http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.2_eng.pdf);
(3) Efficacy Testing of Household Insecticide Products. (http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.3_eng.pdf).

These publications provide standard methods of testing products that seek approval by the WHO for public health use. Such approval is used widely among countries that do not have their own infrastructure for product evaluation. Development of evidence-based and sensible testing methods is important to the military for its own evaluation of products. The U.S. military also must comply with host-nation rules for application of insecticides; therefore, reasonable standards used internationally are important for American forces.

Effective application in a desert environment. The military experience in Iraq showed that hot and dry conditions could interfere with normal vector control procedures. Three large-scale research studies have enabled evaluation of mosquito control products and new techniques in a desert environment. In the first study performed by ARS scientists from Gainesville, Florida, dry ponds in the Coachella Valley in California were used to evaluate the efficacy of ultra-low volume versus thermal fog application of a permethrin-based adult mosquito pesticide under hot, dry, dusty, and windy conditions. The unique properties of the formulation were exploited to examine performance of the pesticide in both oil and water solutions. Results demonstrated high efficacy of the formulation, shown by high mortality in all areas of a 300-foot by 300-foot grid of caged sentinel mosquitoes in all but the oil solution in the thermal fogger, which may have been due to high temperatures from oil combustion affecting the permethrin or causing rapid evaporation.

In the second study, a large hot-arid desert area near the western shore of the Salton Sea in the Coachella Valley was used to evaluate residual barrier treatments of lambda-cyhalothrin, a

residual insecticide similar to bifenthrin, on both a native vegetation perimeter and military camouflage netting using standard and electrostatic sprayers under extreme field conditions of dry heat, dust, and ultraviolet exposure. Camouflage net strips continued to provide a perimeter to form a protected area, and treatments with a standard cold mist blower, an electrostatic spray head, and a handheld electrostatic spray gun were conducted. Efficacy and longevity of barrier treatments were measured with field and lab techniques, including the use of mosquito traps in treated versus untreated areas until efficacy was no longer observed and assessment in the lab with mortality in bioassays on temporal samples of cut vegetation or material using colony-reared mosquitoes. Results from vegetation treatments indicate significant reduction (more than 50 percent) in mosquitoes in field counts and lab assays for up to 1 month for cold mist and 2 months for electrostatic applications. The results of netting treatments indicate significant reduction (more than 90 percent) in mosquitoes in field counts and lab assays for up to 2 months for both cold-mist and electrostatic applications.

In the third study, a large vegetation perimeter was treated with bifenthrin in foam, used in related studies against fleas in rodent burrows. The foam treatment provided 1to 2 weeks of effect against mosquitoes, compared to more than 50 percent mortality for 2 months with water-based treatments. One advantage of the foam treatments was that excess, mixed material could be applied to vegetation or to rodent burrows, solving the problem of disposal of material. These accomplishments identify treatment methods useful for vector control under military conditions in a desert environment and the level and duration of control to be expected so that appropriate resources can be allotted.

Britch, S.C., Linthicum, K., Wynn, W.W., Aldridge, R.L., Walker, T.W., Farooq, M., Dunford, J.C., Smith, V.L., Robinson, C.A., Lothrop, B.B., Snelling, M., Gutierrez, A., Wittie, J., and White, G. 2011. Longevity and efficacy of bifenthrin treatment on desert-pattern U.S. military camouflage netting against mosquitoes in a hot-arid environment. Journal of the American Mosquito Control Association 27(3): 272-279.

Effective applications in sub-Saharan Africa. The most challenging conditions in medical entomology exist in sub-Saharan Africa, where even tourists exposed for short periods to biting insects commonly suffer infections from a variety of tropical, vector-borne pathogens. ARS scientists in Gainesville, Florida, evaluated the efficacy and longevity of barrier sprays and ultra-low volume and thermal fog adulticide treatments in controlling important mosquito and sand fly vectors (as well as other biting and filth fly species) in sub-Saharan Africa. U.S. military desert radar-scattering, ultra-lightweight camouflage netting system material was shown to act as a lethal barrier to vectors (mosquitoes and sand flies) for 18 months after treatment with bifenthrin. Retreatment of the netting material was shown to be a practical option under field conditions. In another study, thermal fog and ultra-low volume studies conducted in Kenya in disease endemic habitats demonstrated that both malathion- and synergized prallethrin/sumithrin-based products were highly efficacious in killing caged populations of adult sand flies. Additionally, wild sand fly populations were monitored using baited light traps and indicated effective population suppression or population displacement following ultra-low volume and thermal fog applications. Repeated trials revealed that thermal fog applications of sumithrin/prallethrin-based pesticides were substantially more efficacious than similar ultra-low volume applications against sand flies. Conversely, ultra-low volume application of malathion consistently outperformed thermal fog against sand flies. This research showed how currently available

materials and equipment can be used to control vectors in sub-Saharan Africa. The information is being integrated into military doctrine.

Britch, S.C., Linthicum, K., Walker, T.W., Farooq, M., Gordon, S.W., Clark, J.W., Ngere, F., N'Gonga, D., and Chepchieng, C. 2011. Evaluation of ULV applications against Old World sand fly (Diptera: Psychodidae) species in equatorial Kenya. Journal of Medical Entomology 48(6): 1145-1159.

Evaluation and design of application equipment. The effective and safe use of insecticides depends as much on the application equipment as on the chemicals themselves. Specifications for existing equipment, the means for evaluation, and the effectiveness of applications must be developed so that results are meaningful. ARS scientists in College Station, Texas, and Gainesville, Florida, have continued cooperative work with the Navy Entomology Center of Excellence at the Jacksonville Naval Air Station in Florida to systematically evaluate commercial powered sprayers for the military. That work has resulted in more realistic evaluation of droplet size, range, and volume, which is essential to knowing how to judge effective application. The spray profile of the new military aerial spray system for mounting on the C-130 was evaluated in a large, high-speed ARS wind tunnel. The scientists developed a smartphone app, Vector Spray, that can be used by military and other personnel in the field to choose the correct sprayer for the job. A series of tests have shown that screened cages often used to bioassay effectiveness of aerosol insecticide applications do not give reliable results because they significantly reduce penetration of the spray to the insects inside. The scientists have developed a model to adjust the results to factors that affect penetration, such as viscosity and wind. This work improves the reliability of pesticide applications by military personnel anywhere in the world. (www.appbrain.com/app/vector-spray/com.echo.usda.ground)

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COMPONENT 3: Veterinary Entomology of Livestock and Poultry

Veterinary entomology is the core of the NP 104 research efforts. Biting arthropods cause three different problems for livestock and poultry. First, they can transmit a wide range of pathogens, many of which are specific to certain species of domestic animals. Examples include bovine babesiosis transmitted by *Rhipicephalus* (*Boophilus*) one-host ticks, equine piroplasmosis transmitted by ticks, and vesicular stomatitis virus transmitted by biting flies. Second, the bites or infestations of the arthropods can be directly damaging to the animal. Stable flies as pests of cattle are a prime example. Finally, insects can spread organisms such as *Salmonella enteriditis* in poultry, which threaten food safety.

This National Program has been involved in a series of conquests of major livestock pests, including the cattle bot fly, the sheep mange mite (formerly an important pest of cattle), and cattle lice. Research continues on cattle fever ticks and screwworm flies, pests that have been eradicated in the United States but that require constant operational activity to maintain that success. Other pests in the program are considered the most important current threats to animal agriculture in the United States—stable flies, house flies, horn flies, mosquitoes, biting midges, phlebotomine sand flies (possibly an as-yet-undocumented threat), and vectors that are actual or potential invasive species. The efforts in the medical components provide benefits for veterinary entomology and vice versa, often to the point that the same accomplishment could apply to Components 1, 2, and 3.

As mentioned above, research in veterinary entomology has considerable relevance for medical entomology, both for control of zoonotic vectors and for development of products with uses for protection of humans as well as animals. In contrast to the medical entomology programs, much of the government interaction has been with USDA Animal and Plant Health Inspection Service (APHIS) and the Food and Drug Administration (FDA). ARS works directly with APHIS on both the Screwworm Eradication Program and the Cattle Fever Tick Eradication Program, each of which is an operational program that protects the United States from an estimated $10 billion dollars in annual damage. The FDA licenses anti-tick vaccines, injectable insecticides, and insecticides fed in mineral blocks, all products that ARS has been involved in developing and testing. Because of the large commercial interests, stakeholders in the cattle industry have had close association with elements of the veterinary entomology program, and NP 104 researchers maintain close cooperation with the pork, equine, and poultry industries.

The veterinary entomology portion of the program has also worked extensively with industrial partners. Among the products under development are repellents for application to equines and cattle, products for control of biting midges to protect deer, devices to treat ticks on wild deer, ivermectin bait blocks for tick control with possible effectiveness against flies, and anti-tick vaccines.

The accomplishments for Component 3 are aligned under the nine Problem Statements noted in the Action Plan.

PROBLEM STATEMENT 3A**:** *The Rhipicephalus (Boophilus) one-host tick transmits bovine babesiosis (Texas cattle fever) among cattle. An extensive effort started in the early 1900s culminated in eradication of this tick from the entire U.S. in 1943, eliminating the threat of Texas cattle fever to the beef industry. In recent years, the cattle fever tick has been expanding its range into the southern counties of Texas despite the continuing efforts by APHIS (Cattle Fever Tick Eradication Program) to maintain the control area. The expansion in range is probably the result of the increase in numbers of an alternative host, the white-tailed deer. In addition, feral exotic ungulates (e.g., axis deer) may contribute to the tick infestations. At the current time, the dollar value of the ungulate hunting business is greater than the value of cattle in parts of this region, making landowners reluctant to limit the ungulate herds. Although laboratory tests indicate that introduced ticks are usually susceptible to acaricides, dipping treatments of infested cattle herds seem to be failing more often. Unless this situation is understood and controlled, the cattle industry throughout the southern United States could be threatened with a disease that has been controlled for over 60 years.*

The following location has research projects addressing the issue identified under Problem Statement 3A:

- Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas. (This laboratory has a significant satellite function at Moore Air Base in Mission, Texas.)

Problem Statement 3A: Select Accomplishments

Discovery of the synergistic effect of permethrin and amitraz mixtures. The cattle fever tick is kept out of the United States with the cooperation of Mexico by dipping cattle that enter the country. The appearance of resistance to acaricides in Mexico raises concern that these procedures will no longer be effective. ARS scientists in Kerrville, Texas, have found synergism between permethrin and amitraz against the southern cattle tick, the first report of such synergism in ticks. This synergistic mixture might be useful against resistant populations. The existence of strong synergistic effects between acaricides with different modes of action may be an indication that other mixtures would also be useful. Some products not registered in the United States contain mixtures of acaricides. These products could offer hope for controlling cattle fever ticks if current methods fail.

Barre, N., Li, A.Y., Miller, R.J., Gaia, H., Delathiere, J., Davey, R.B., and George, J.E. 2008. In vitro and in vivo evaluation of deltamethrin and amitraz mixtures for the control of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in New Caledonia. Veterinary Parasitology 155:110-119.

Li, A.Y., Miller, J.A. and Klavons, J.A. 2008. Release of piperonyl butoxide and permethrin from synergized ear tags on cattle and effects on horn fly mortality. Journal of Economic Entomology 101(5):1697-1703.

Chen, A.C., He, H., and Davey, R.B. 2007. Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks. Veterinary Parasitology 148:379-383.

Anti-tick vaccines and the tick genome. Efforts to sequence the genome of the cattle fever tick, *Rhipicephalus microplus*, have shown that this species has very large areas of repeating sequence that make assembly a challenge. In spite of these challenges, work to date by ARS scientists in Kerrville, Texas, has been useful in distinguishing populations of the tick, in evaluating the suitability of tick populations as parasites of particular populations of cattle, and, especially, in the discovery of antigens for use as anti-tick vaccines. These targets are associated with the salivary gland proteins, anticoagulants secreted by ticks during feeding, and tick ovarian proteins. Extensive work toward describing the complicated genome of this tick leverages the biochemical work by identifying the genes that could be used to produce antigens for the vaccine products. Trials with an older vaccine based on gut basement membrane proteins (Bm86, trade name GAVAC) were conducted in cooperation with APHIS. These trials required importation of vaccine from Cuba and testing of lots at Plum Island, New York, a process that required many months of effort. The trials showed that the vaccine had virtually no effect on the southern cattle fever tick, *Rhipicephalus microplus*, but was very effective against the cattle fever tick, *Rhipicephalus annulatus*. In the meantime, efforts based on exploration of the sequenced parts of the tick genome for sequences corresponding to common immunostimulatory epitopes produced a series of candidate antigens. Tested in cooperation with Brazil's agricultural research service, EMBRAPA, some of these candidates were much more effective against *Rhipicephalus microplus*. An effective anti-tick vaccine would not only have a large international market for management of one-host ticks, but it would also strengthen the quarantine zone of southern Texas that keeps these ticks out of the United States.

Lew-Tabor, A.E., Moolhuijzen, P.M., Jones, M.E., Kurscheid, S., Rodriguez Valle, M., Jarrett, S., Minchin, C.M., Jackson, L.A., Jonnson, N.N., and Guerrero, F. 2010. Suppressive subtractive hybridization analysis of Rhipicephalus (Boophilus) microplus larval and adult transcript expression during attachment and feeding. Veterinary Parasitology 167:304-320.

Freeman, J.M., Davey, R.B., Kappmeyer, L.S., Kammlah, D.M., and Olafson, P.U. 2010. Bm86 midgut protein sequence variation in south Texas cattle fever ticks. Parasites and Vectors 3:article 101.

Temeyer, K.B., Olafson, P.U., and Miller, R. 2009. Genotyping mutation in BmAChE3: A survey of laboratory and Mexican strains of Rhipicephalus (Boophilus) microplus that are resistant or susceptible to coumaphos. Journal of Medical Entomology 46(6):1355-1360.

Oral ivermectin to control cattle fever ticks. Cattle ranchers in southern Texas are subject to laws that require expensive and inconvenient handling of their animals if cattle fever ticks are found. These rules are necessary to prevent the reinfestation of the rest of the United States, but producers have difficulty maintaining their operations when they either have to vacate pastures for 9 months or treat the cattle on infested pastures every 2 weeks. ARS scientists in Kerrville, Texas, demonstrated that pastured cattle fed ad lib liquid molasses containing ivermectin, a systemic parasiticide and acaricide, resulted in serum concentrations of the drug sufficient to control all of the fever ticks feeding on the animals (>10 ppb). This finding led to development of a mineral bait block product in a tub that also controls ticks on cattle. The product is being developed by an industrial partner and undergoing licensing by FDA. Once available, the product may replace biweekly dipping of cattle currently required on infested pastures.

Davey, R.B., Pound, J.M., Miller, J.A., and Klavons, J.A. 2010. Therapeutic and persistent efficacy of a long-acting (LA) formulation of ivermectin against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and sera concentration through time in treated cattle. Veterinary Parasitology 169(1-2):149-156.

Controlling cattle fever ticks on deer. The increase in deer populations in southern Texas has presented a daunting challenge to those responsible for preventing cattle fever tick reinfestation in the United States. ARS scientists in Kerrville, Texas, and their collaborators at Texas A&M University, with funding from NIFA, have shown that deer can maintain cattle fever ticks and that they are commonly infested. A complicating problem is that deer are important to producers, who depend on them for primary or supplementary income derived from hunting.

Originally developed for Lyme disease control, the ARS 4-Poster deer treatment bait station applies acaricide to deer attracted to corn bait and has been adapted for use in southern Texas. Field trials of technologies under development to prevent feral swine and javelina from accessing and destroying the bait stations were completed, with varying degrees of positive results. Initial attempts to see if access to the 4-Posters could be prevented by attaching the bait stations to metal posts to raise them off the ground proved insufficient. In fact, the swine were so large that they could place both front legs on the devices and use their snouts to dislodge the lids that were held tightly with heavy rubber straps. However, an 18-inch-high, 30-foot-diameter welded wire 4x4 inch mesh circular barrier fence was constructed around the 4-Poster, and two strands of electric fence wire were added to the exterior approximately 4 inches below the top edge of the fence. The taller deer sniffed the top of the fence before jumping inside; however, the swine, having shorter legs, contacted and were shocked by the electric fence wire, then rapidly retreated.

In addition, field trials were done with the ARS-designed and recently commercialized 2-Poster deer treatment feeder adapter to evaluate ruggedness of design and suitability for pesticide application. These adapters were developed to retrofit cylindrical and rectangular feed-chute type protein feeders commonly used by ranchers to feed deer. The 2-Posters are now used by the Texas Animal Health Commission as one of the tools to help keep cattle fever ticks out of the United States.

Carroll, J.F., Pound, J.M., Miller, J.A., and Kramer, M.H. 2008. Reduced interference by gray squirrels with 4-poster deer treatment bait stations by using timed-release bait. Journal of Medical Entomology 33(2):325-332.

Daniels, T.J., Falco, R.C., Mchugh, E.E., Vellozzi, J., Boccia, T., Denicola, A.J., Pound, J.M., Miler, J.A., George, J.E., and Fish, D. 2009. Acaricidal treatment of white-tailed deer to control *Ixodes scapularis* (Acari: Ixodidae) in a New York Lyme disease-endemic community. Vector-Borne and Zoonotic Diseases 9(4), 381-387.

PROBLEM STATEMENT 3B**:** *Stable flies (*Stomoxys *species) are not adequately controlled on most farms and there is a threat of importation of other, related species of flies.*

The following locations have research projects addressing the issue identified under Problem Statement3B:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.
- Agroecosystem Management Research Unit, Lincoln, Nebraska.
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Problem Statement 3B: Select Accomplishments

Genomic studies on stable flies. Complete sequencing of key arthropod species has formed a library of gene sequences that has been essential in the process of unlocking the identification of genes in many other species of pests. ARS scientists in Kerrville, Texas, have used more streamlined methods of gene identification to find 21 genes associated with host seeking and egg laying in stable flies, including the first olfactory and taste receptors to be reported for this significant livestock pest. The researchers were also able to find the specific change in a gene that resulted in resistance to the commonly used veterinary pesticide, permethrin. Through laboratory selection, University of Florida researchers working with ARS in Gainesville, Florida, previously obtained a 15-fold resistance to permethrin in a strain of stable flies. The ARS scientists identified a mutation in the stable fly sodium channel gene that associates with the observed resistant phenotype. The findings of this research could lead to highly specific molecular pesticides, design of chemicals that alter stable fly behavior, and methods for field detection of insecticide resistance. Work on particular products that makes use of these discoveries is going on at ARS laboratories in Lincoln, Nebraska; Kerrville; and Gainesville.

Olafson, P.U., Pitzer, J.B., and Kaufman, P.E. 2011. Identification of a mutation associated with permethrin resistance in the para-type sodium channel of the stable fly (Diptera: Muscidae). Journal of Economic Entomology 104(1):250-257.

Ascunce, M.S., Yang, C.C., Geden, C.J., and Shoemaker, D.D. 2009. Twenty-three new microsatellite loci in the stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Molecular Ecology Resources 9:271-273.

Economic impacts of stable flies. Stable flies are known to be a major livestock pest, but it has been difficult to estimate their economic impact. ARS scientists in Lincoln, Nebraska, developed a dynamic and explicit model to estimate the economic impact of stable flies on the U.S. cattle industry. Results indicated that production losses total $872 million to the dairy industry, $114 million to cow/calf operations, $1.3 million to pastured stocker cattle, and $197 million to feeder cattle, for a total impact of nearly $2.5 billion in 2008. This model provides the data needed for producers to include cost-benefit analyses when making decisions concerning the cultural changes needed to reduce stable fly populations on a regional scale. These calculations, published in the open literature, should help industry focus more efforts against stable flies.

Taylor, D.B., Moon, R.D., and Mark, D.R. 2012. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. Journal of Medical Entomology 49(1): 198-209. Available: http://dx.doi.org/10.1603/ME10050.

New chemical methods to protect cattle from stable flies. The use of round hay bales for feeding cattle often creates highly productive larval sites for stable flies in the soil where animals mix together urine and uneaten fodder. These larval sites have presented a big challenge to any kind of chemical control. ARS scientists in Lincoln, Nebraska, showed that cyromazine, a growth regulator registered for house flies, was highly effective in this situation. The scientists have also been working with natural products to modify behavior by stable flies. Tests of a botanical-based stable fly repellent from the essential oil of catnip (*Nepeta cataria*) have shown strong repellency against adult stable flies. The water and oil-based catnip oil formulations can deter stable fly biting on cattle for 5 to 7 hours in field conditions. In addition, encapsulated and starch formulations of catnip oil can effectively reduce stable fly oviposition over 95 percent and inhibit larval growth up to 90 percent. Catnip oil products have been shown to repel biting stable flies and ovipositing flies from cattle, and they apparently do not irritate the animals' skin. Such products may be very useful in protecting cattle, eliminating difficult larval sites, and providing the "push" in push-pull strategies to control stable flies. Product development with an industry cooperator is underway.

FIGURE 3: An ARS technician applies cyromazine for stable fly control in a test for efficacy near Lincoln, Nebraska.

Taylor, D.B., Friesen, K.M., Zhu, J.J., and Sievert, K. 2012. Efficacy of cyromazine to control immature stable flies (Diptera: Muscidae) developing in winter hay feeding sites. Journal of Medical Entomology 105:726-731.

Zhu, J.J., Li, A.Y., Pritchard, S., Tangtrakulwanich, K., Baxendale, F.P., and Brewer, G. 2011. Contact and fumigant toxicity of a botanical-based feeding deterrent of the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) Journal of Agricultural and Food Chemistry 59: 10394-10400. Available: http://dx.doi.org/10.1021/jf2016122.

Liu, S.S., Li, A.Y., Witt, C.M., and Perez De Leon, A.A. 2011. Immunohistological localization of serotonin in the CNS and feeding system of the stable fly S*tomoxys calcitrans* L. (Diptera: muscidae). Archives of Insect Biochemistry and Physiology 77(4):199-219.

PROBLEM STATEMENT 3C**:** *The housefly (Musca domestica) is not adequately controlled on most farms.*

The following locations have research projects addressing the issue identified under Problem Statement 3C:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Agroecosystem Management Research Unit, Lincoln, Nebraska.
- Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Problem Statement 3C: Select Accomplishments

Salivary gland hypertrophy virus of house flies. The salivary gland hypertrophy virus (SGHV) belongs to a novel group of viruses that infect salivary glands and block egg production in their hosts. A field survey by ARS scientists from Gainesville, Florida, demonstrated that this virus was widely distributed in fly populations on Danish dairy farms, with maximum infection rates of about 10 percent. Transmission studies indicated that the virus was specific to house flies; no infections were found in other sympatric flies (e.g., *Scatophaga stercoraria*, stable fly, face fly, blow flies, or *Eristalis tenax*) that were injected with SGHV. Danish virus isolates had somewhat higher virulence in lab bioassays than did the standard Florida SGHV strain. Transmission tests also demonstrated that virus particles are stored in the crop of the fly, and the live virus is deposited in regurgitated material and fecal spots. These results, along with previous studies on surface treatments, suggest that the most effective method for deploying the virus as an operational control tool may be treatment of fly resting sites.

Geden, C.J., Steenberg, T., Lietze, V., and Boucias, D.G. 2011. Salivary gland hypertrophy virus of house flies in Denmark: prevalence, host range, and comparison with a Florida isolate. Journal of Vector Ecology 36(2):231-238.

Geden, C.J., Garcia-Maruniak, A., Lietze, V., Maruniak, J., and Boucias, D.G. 2011. Impact of house fly salivary gland hypertrophy virus (MdSGHV) on a heterologous host, *Stomoxys calcitrans*. Journal of Medical Entomology 48(6):1128-1135.

Prompiboon, P., Lietze, V., Denton, J., Geden, C.J., Steenberg, T., and Boucias, D.G. 2010. *Musca domestica* salivary gland hypertrophy virus, a globally distributed insect virus that infects and sterilizes female houseflies. Applied and Environmental Microbiology 76(4):994-998.

Pyriproxyfen for larval fly control. House fly larvae (maggots) are notoriously difficult to control with chemicals because of the thick, organic nature of their developmental sites. ARS scientists in Gainesville, Florida, developed the first effective and environmentally safe treatment for house fly larvae using pyriproxyfen. This chemical imitates a hormone found only in insects and is so concentrated in its effect that adult flies can carry enough residual material to deposit an effective dose while laying eggs. This work may result in a product that not only kills maggots, but also takes advantage of the flies themselves to apply the material to larval sites.

Geden, C.J. and Devine, G.J. 2012. Pyriproxyfen and house flies (Diptera: Muscidae): effects of direct exposure and autodissemination to larval habitats. Journal of Medical Entomology 49: 606-613.

PROBLEM STATEMENT 3D**:** *The horn fly (Haematobia irritans) can be a damaging pest of cattle in the United States.*

The following locations have research projects addressing the issue identified under Problem Statement 3D:

- Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.
- Agroecosystem Management Research Unit, Lincoln, Nebraska.
- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Problem Statement 3D: Select Accomplishments

Foundational work on horn fly biology. Horn flies are a severe pest of cattle, and they live a semi-ectoparasitic existence closely associated with the cattle themselves. These life habits suggest that horn flies might be susceptible to genetic approaches for control because the effective population size is likely to be limited. During the first part of a project to create a transgenic strain of screwworm, ARS scientists in Kerrville, Texas, used horn flies as a model, with the intention of making progress towards developing a better genetic understanding of that pest as well. The scientists completed an extensive, annotated expressed sequence tag library for horn fly, which should lead to a better understanding of sexing mechanisms of the fly and will provide a resource for further work on this pest. As part of this research, the scientists examined the physiology of horn fly neurotransmitters using immunocytochemistry. They were able to identify potential targets for new insecticides. This information will not only be useful for the development of new chemicals for control of horn flies, but also for the design of genetic control programs.

Foil, L.D., Guerrero, F.D., and Bendele, K.G. 2010. Detection of target site resistance to pyrethroids and organophosphates in the horn fly using multiplex polymerase chain reaction. Journal of Medical Entomology 47(5): 855-861.

Cupp, M.S., Cupp, E.W., Zhang, D., Yue, X., Todd, L., Panangala, V., Navarre, C., and Whitley, E. 2009. Salivary gland thrombostasin isoforms differentially regulate blood uptake of horn flies fed on New Zealand white rabbits. Journal of Medical Entomology 46(2): 351-357.

Guerrero, F D., Dowd, S.E., Djikeng, A., Wiley, G., Macmil, S., Saldivar, L., Najar, F., and Roe, B.A. 2009. A database of expressed genes from *Cochliomyia hominivorax* (Diptera: Calliphoridae) Journal of medical entomology 46(5): 1109-1116.

Systemic doramectin for horn fly control. The horn fly continues to be an economically important pest of cattle in the United States and elsewhere in the world. ARS scientists in Kerrville, Texas, demonstrated that 10 ppm of doramectin in molasses provided ad lib to pastured cattle completely inhibited horn fly development in the manure. Their findings showed that providing the medicated molasses for 3 weeks and not retreating until fly populations returned to 100 flies per animal provided an effective, efficient, and inexpensive means of maintaining populations below the economic threshold of 200 flies per animal. Reinfestation was attributed mainly to movement of flies from adjacent untreated cattle. These findings show

that a medicated protein/molasses block currently under development for control of cattle fever ticks may also help control horn flies.

Lohmeyer, K.H., Miller, J.A., Pound, J.M., and Klavons, J.A. 2009. A sustained release gel formulation of Doramectin for control of lone star ticks (Acari: Ixodidae) and horn flies (Diptera: Muscidae) on cattle. Journal of Economic Entomology 102(2): 804-808.

Commercial system for application of insecticide to cattle. The timing of pesticide application to cattle depends on the kinds of pests present during that season. Producers have many concerns and using the right product at the right time can be a challenge. ARS scientists in Kerrville, Texas, developed combinations of insecticides and synergists packaged into color-coded paint balls. The use of paint balls eliminates the need to put cattle into chutes. Color-coding the correct mixture of chemicals for treatment in each season gives producers a simple system for applying the right insecticide at the right time for horn flies. This system is under commercial development by an industrial partner.

Li, A.Y., Ross, D. and Perez De Leon, A.A. 2011. Evaluation of the VetCap treatment method for horn fly control in cattle. International Journal of Applied Research in Veterinary Medicine 9(2):198-203.

PROBLEM STATEMENT 3E**:** *The screwworm fly (Cochliomyia hominivorax) has been eliminated from North America thanks to a program of detection, animal treatment, and mass release of sterile male flies. The current status of APHIS' Screwworm Eradication Program is maintenance of a barrier zone in eastern Panama, protecting the entire continent to the north. This program involves a continuous obligation of funding in order to maintain the barrier and protect the U.S. from this devastating pest of mammals. Expansion of the program to the Caribbean and South America is a possibility, but not currently planned or funded by the U.S. government. Various governments and NGOs discuss such expansion and have funded several efforts. ARS continues to perform research that improves the efficiency of the rearing, release, and control of screwworm flies.*

The following locations have research projects addressing the issue identified under Problem Statement 3E:

- Screwworm Research Unit, Pacora, Panama (based out of the Knipling-Bushland U.S Livestock Insects Research Laboratory).
- Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.

Problem Statement 3E: Select Accomplishments

Saving money through changes in rearing. The screwworm fly is a dramatically damaging pest of the Western Hemisphere that lays eggs that hatch into flesh-eating maggots on mammals, including humans and livestock. The screwworm fly had been seen as far north as the mid-western United States, but was eradicated from North and Central America by systematic distribution of sterile male flies by USDA. These flies must be reared, irradiated, and distributed, a process that currently costs the U.S. government approximately $10 million per year to establish a barrier of sterile flies between infested areas in South America and Panama.

ARS scientists in Pacora, Panama; Kerrville, Texas; and Fargo, North Dakota, continue to improve the efficiency of the process by applying new technology. This year, automated cryopreservation equipment was installed at the rearing plant, which eliminates the need to continuously rear a back-up colony and reference strains. Researchers identified chemicals that attract the flies to a site for egg-laying. Those chemicals will improve the rearing process by coordinating egg laying by colony flies and by providing a better means of treating flies in small outbreaks. The scientists were able to genetically transform multiple lines of the flies, incorporating a marker protein and a cassette of DNA that induces the flies to produce only males. These accomplishments will reduce costs and increase reliability of rearing. A strain of flies that produces only males will save significant money in rearing costs, as well as reducing the level of radiation required to sterilize released flies. Although this accomplishment falls after the period covered by this report, ARS scientists demonstrated in September 2012 that one of the transformed strains expressed the conditional female-lethal trait intended by research.

New strain of screwworm introduced for production of sterile males. Screwworm flies are kept out of Central and North America by distribution of sterile male flies in a band of territory in eastern Panama, forming a barrier against screwworm populations in South America. Using the sterile insect technique to eradicate screwworms has been highly successful, but periodically the Mexico–U.S. Commission for Eradication of Screwworms (COMEXA) requires a new strain for use in the mass rearing facility. The nature of mass rearing programs using the sterile insect technique involves periodically changing the strain because of excess "colony adaptation," as suggested by poor performance in the field. ARS scientists in Pacora, Panama, and their collaborators at COMEXA developed a new strain of screwworm fly, called Jamaica-06, and transferred it to the COMEXA mass rearing facility in Tuxtla Gutierrez, Chiapas, in Mexico. This marked the first joint effort on strain development between the ARS and COMEXA**.** Constraints to use of this strain in the eradication program relate to the ability of COMEXA to sufficiently "domesticate" the strain so that it is readily mass produced yet effective in the field. COMEXA personnel are now capable of producing new strains with consultation from ARS. ARS entomologists in Pacora, Panama, performed a field study that showed that the new production strain, Jamaica-06, is just as effective as the strain that has been in use for more than 5 years.

Modifications to screwworm rearing medium to reduce ammonia levels. A new sterile insect production plant in Panama was designed for use of an acrylamide gel-based diet that absorbed nitrogenous waste products of the larvae, but that created waste disposal problems. After design of the plant, ARS scientists developed a cellulose-based diet that avoided those problems and was better for the flies as well. However, the cellulose-based diet did not absorb nitrogenous wastes, so that a higher level of gaseous ammonia was created from the larval rearing process. Those levels exceeded the occupational threat limit value for hundreds of workers in the plant. ARS scientists in Pacora, Panama, were called upon to perform rapid-response research to determine how best to reduce ammonia production in larval medium, a problem that had become a major impediment to operating the new Panama plant. The selection of a medium that produces a minimum of ammonia has enabled APHIS to plan on scaling up the Panama plant to full production. Changes to the medium included inclusion of chemicals that absorb ammonia and use of some acrylamide mixed with cellulose. As a result of this research, APHIS saved tens

of millions of dollars that would have been required to make changes to the air handling system of the production plant.

Chaudhury, M.F. and Skoda, S.R. 2009. Diet pH, and viscosity affect development and survival of screwworm larvae (Diptera:Calliphoridae). Journal of Economic Entomology 102(2):799-803.

Chaudhury, M.F. and Skoda, S.R. 2007. A cellulose fiber-based diet for screwworm (Diptera: Calliphoridae) larvae. Journal of Economic Entomology 100(1):241-245.

Release technologies analyzed to improve sterile fly dispersal. Sterile male screwworm flies are expensive to produce, and the production capacity of a new plant in Panama is limited, so there is a need to use flies as effectively as possible. ARS researchers in Kerrville, Texas, and Pacora, Panama, reviewed and analyzed release technologies to improve screwworm fly dispersal relative to where, when, and how many sterile flies are released in the barrier zone located in eastern Panama. Quantitative calculations were based on screwworm biology and modeling of results, as well as application of global information systems. Recommendations to match the number of flies released to the calculated populations of flies on the ground were presented to the Panama–U.S. Commission for Eradication of Screwworm (COPEG). Implementation of those recommendations would (1) result in updated equipment on board dispersal aircraft, (2) strategically reduce the number of flies dispersed, and (3) save up to $1 million annually. Recommended improvements to the navigational software and equipment are currently being implemented by APHIS International Services and COPEG, the agencies that operate the sterile male barrier zone.

Alternative key ingredient for screwworm rearing. Mass rearing of sterile screwworm flies is often disrupted by inadequate supplies of key ingredients for rearing, particularly spray-dried blood. ARS researchers in Pacora, Panama, found that defibrinated, fresh bovine blood-based diets were equivalent to the current standard, spray-dried blood-based diet. The researchers also determined that the use of sodium citrate, commonly used as an anticoagulant, was detrimental to developing screwworms. Results communicated directly to COPEG have increased the variety of components for screwworm diets, providing alternatives in case of shortages of some ingredients and allowing purchase of the least expensive alternatives.

Chaudhury, M.F., Skoda, S.R., and Sagel, A. 2011. Solidifying agent and processing of blood used for the larval diet affect screwworm (Diptera: Calliphoridae) life-history parameters. Journal of Economic Entomology 104(3):1103-1107.

PROBLEM STATEMENT 3F**:** *Mosquitoes bite domestic animals, but little is known about their potential to cause decreases in yield; control is difficult in rural settings.*

The following locations have research projects addressing the issue identified under Problem Statement 3F:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas.

Problem Statement 3F: Select Accomplishments

Attractants to survey and kill adult mosquitoes. Mosquito attractants can be important tools for surveillance and in the potential development of lethal baits. ARS researchers in Gainesville, Florida, have developed oviposition attractants after analysis of chemicals from oviposition sites. The chemicals can be used to enhance surveillance devices, particularly to standardize attractiveness between traps rather than using fermented media. The attractants, for which ARS is seeking a patent, may also be important for broadening the effectiveness of sugar-based baits developed by ARS. A range of insecticides have been evaluated for effectiveness in baits and other devices that are acceptable to mosquitoes but that exclude bees. Attractive, lethal baits for mosquitoes would be particularly suitable in an agricultural setting where larval control may be impractical and the locations of hosts (i.e., livestock) are well defined.

Kline, D.L., Allan, S.A., Bernier, U.R., and Welch, C.H. 2007. Evaluation of the enantiomers of 1-octen-3-ol and 1-octyn-3-ol as attractants for mosquitoes associated with a freshwater swamp in Florida, USA. Medical and Veterinary Entomology 21(4) 323.

Behavior of mosquitoes in response to a treated surface. Barrier treatments are a way to disrupt movement of mosquitoes toward hosts and to generally reduce an adult population that escaped larval treatment. This method of treatment could be important to protect livestock and poultry barns as well as pastures in which animals are concentrated. ARS researchers in Gainesville, Florida, compared the behavioral responses of *Aedes, Anopheles,* and *Culex* mosquitoes to surfaces treated with three different pyrethroids. Each pyrethroid tested affected the numbers of landings and the contact duration of each mosquito species differently. These data provide the basis for selection of appropriate pesticides for treatment of barriers or surfaces to enhance numbers and duration of contacts to maximize mosquito mortality caused by the insecticide. These results have influenced the selection of insecticides used for barrier treatment trials and help to explain the variability in effectiveness of barrier treatments against various species of mosquitoes.

Cooperband, M.F., Golden, F.V., Clark, G.G., Jany, W., and Allan, S.A. 2010. Prallethrin-induced excitation increases contact between sprayed ultra-low-volume droplets and flying mosquitoes (Diptera: Culicidae) in a wind tunnel. Journal of Medical Entomology 47(6):1099-1106.

Viral biological control of mosquitoes. The use of viruses to control larval mosquitoes has been suggested for decades, but practical application has been elusive. The method has appeal for agricultural settings because the viruses are very specific against mosquitoes and are possibly self-replicating, qualities that would make them an economical and environmentally safe method of control. ARS scientists in Gainesville, Florida, have been researching the biology of mosquito viruses for many years, identifying new kinds and discovering during the last reporting period that calcium-potassium divalent cation concentrations in water determine whether or not some viruses infect mosquito larvae. During this reporting period, the scientists identified the major structural proteins of a baculovirus that infects and kills mosquitoes. Forty-four structural proteins and their genes have been identified, some of which play crucial roles in infectivity, host specificity, and virulence. This work has opened up a range of new possibilities for mosquito control. Four proteins crucial for oral infections have been found to be promising new targets for

development as biocontrol tools. It is now possible to investigate these and other viral proteins at the molecular level and understand how they exploit essential metabolic processes of the mosquito host. These results show that viral control of mosquitoes may require additional management to be effective.

Liu, B., Becnel, J.J., Zhang, Y., and Zhou, L. 2011. Defensive reaper induction of mx and apoptosis in mosquito midgut cells as an innate immune response to baculovirus infection. Cell Death and Differentiation 18:1337-1345. DOI: 10.1038/CDD.2011.8.

Zhao, L., Becnel, J.J., Clark, G.G., Linthicum, K., Chen, J., and Jin, X. 2010. Identification and expression profile of multiple genes in response to magnesium exposure in *Culex quinquefasciatus* larvae. Journal of Medical Entomology 47(6):1053-1061.

Becnel, J.J. 2007. Current status of deltabaculoviruses, cypoviruses, and chloriridoviruses pathogenic for mosquitoes. Virologica Sinica 22: 117-127.

Tools for IPM of the mosquito vectors of West Nile virus and other livestock diseases. West Nile virus is principally transmitted in the United States by a few species of *Culex*. Control of larvae with bacterial toxins derived from *Bacillus thuringiensis israelensis* (*Bti*) is the principle method used because of the environmental safety of the product. However, *Bti* has its limitations, including a requirement for regular retreatment, limited shelf life, inability to kill non-feeding immature stages (i.e., late fourth instars and pupae), and reduced efficacy at low temperatures. *Bacillus sphaericus* (*Bs*) is another bacterium containing proteins toxic to mosquitoes, but it recycles following application and has better effectiveness than *Bti* in water with high organic content. *Bs* has the disadvantage that local resistance often develops following application, and it does not affect all kinds of mosquitoes. A few products have come on the market that combines the two bacteria. ARS scientists in Gainesville, Florida, worked with authorities in Connecticut to perform trials of one of these products, VectoMax. Their findings suggest that a second treatment is likely needed to reduce mosquitoes to relatively low numbers in catch basins. Another test in catch basins in Connecticut focused on an evaluation of Natular, a product containing the powerful growth regulator, pyriproxyfen.

Other work was performed on the importance of natural enemies on control of West Nile vectors in the Northeast and likely changes in species distribution brought about by the expansion of *Aedes japonicus,* introduced into the United States in 1998. Combined with virus surveillance of wild mosquitoes and transmission studies showing the high susceptibility of *Culex tarsalis* to the highly pathogenic form of West Nile virus, this work, which was carried out in partnership with the Connecticut Agricultural Experiment Station, made progress toward development of better methods for control of mosquito vectors in the Northeast.

Konrad, S.K., Miller, S.N., Reeves, W.K., and Tietze, N.S. 2009. Case study of a degree-day model of West Nile virus risk: Santa Clara County, California, USA. Vector-Borne and Zoonotic Diseases Vol. 9: 267-274

Risk assessment of Rift Valley fever. If Rift Valley fever were introduced into the United States, the country would experience severe disruption of its livestock industry, as well as a major public health threat. ARS and U.S. Army scientists collaborated to evaluate the competence of native American mosquitoes for transmission of Rift Valley fever virus. Species evaluated

included *Culex erraticus, Cx. erythrothorax, Cx. pipiens, Cx. quinquefasciatus, Cx. tarsalis, Aedes dorsalis, Aedes vexans,* and *Anopheles quadrimaculatus* (as well as the biting midge, *Culicoides sonorensis*). *Cx. tarsalis* was found to be a competent vector of the virus, while *Cx. erythrothorax, Cx. pipiens, Cx. erraticus,* and *Aedes dorsalis* were found to be moderately competent. Only *Anopheles quadrimaculatus* and *Culicoides sonorensis* were found not to be competent vectors of Rift Valley fever virus. Two populations of *Aedes vexans* were found to differ in their vector competence, indicating the necessity for further vector population investigations. The results are interesting biologically in that a virus with limited geographic range in Africa appears to be pre-adapted for transmission by North American mosquitoes. More practically, the results show that North America is fully susceptible to the introduction of Rift Valley fever virus, just as it was to West Nile virus.

Additionally, Rift Valley fever virus infection of house flies, *Musca domestica* L., and stable flies, *Stomoxys calcitrans* (L.), by ARS scientists showed that neither species supported the replication of the disease, even after intrathoracic inoculation. However, *S. calcitrans* was able to mechanically transmit Rift Valley fever virus to susceptible hamsters after probing on infected hamsters with high viral titers. Therefore, *S. calcitrans*, because of its close association with domestic animals that serve as amplifying hosts of Rift Valley fever virus, should be considered a possible mechanical vector of the disease, and it may contribute to the rapid spread of a Rift Valley fever outbreak. Other *Stomoxys* species present in Africa and elsewhere may play similar roles.

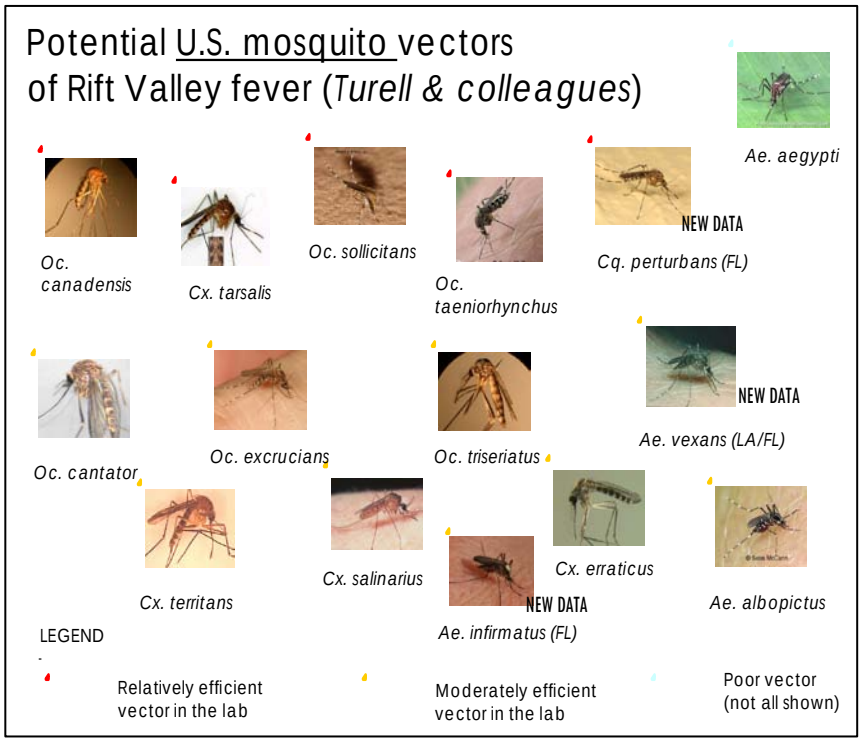

FIGURE 4: Mosquito populations tested for Rift Valley fever virus vector competence in the lab, indicating level of potential vector competence. Of particular interest is apparent geographic variation in vector competence within species, such as *Ae. vexans*.

In the past year, ARS efforts were directed towards improving a spatially explicit GIS-based model that identifies the temporal and spatial patterns that determine risk of establishment of Rift Valley fever virus in the United States. A model developed by ARS and NASA can now incorporate the most recently released weather data and model temperature-based risk across the United States in near real time. The model works on three sources of data: (1) interpolated temperature produced by NOAA for the United States at a 0.5x0.5 degree resolution, (2) city-

and town-based data from over 2,800 weather stations that report on a daily basis, and (3) the short-term weather forecast data produced by NOAA. These data have been used to produce a functional Web-based platform that shows the daily risk for disease transmission of arthropod-borne illness such as West Nile virus and Rift Valley fever virus in the lower 48 states.

Turell, M.J., Dohm, D.J., Geden, C.J., Hogsette, Jr, J.A., and Linthicum, K. 2010. Potential for stable flies and house flies (Diptera: Muscidae) to transmit Rift Valley fever virus. Journal of the American Mosquito Control Association 26:445-448.

Konrad, S.K., Miller, S.N., Reeves, W.K. 2010. A spatially explicit degree-day model of Rift Valley fever transmission risk in the continental United States. Geojournal: 10.1007/s10708-010-9338-x

Linthicum, K., Anyamba, A., Britch, S.C., Chretien, J., Erickson, R.L., Small, J., Tucker, C.J., Bennett, K.E., Mayer, R.T., Schmidtmann, E.T., Andreadis, T.G., Anderson, J.F., Wilson, W.C., Freier, J., James, A., Miller, R., Drolet, B.S., Miller, S., Tedrow, C., Bailey, C., Strickman, D.A., Barnard, D.R., Clark, G.G., and Zou, L. 2007. A Rift Valley fever risk surveillance system for Africa using remotely sensed data: Potential for use on other continents. Veterinaria Italiana 43(3): 663-674.

PROBLEM STATEMENT 3G: *Biting midges transmit viruses to cattle, sheep, and deer; the midges are often numerous enough to create a significant annoyance. Recent expansion of the transmission of blue-tongue virus and emergence of Schmalenberg virus in Europe suggest that biting midges may have an expanding importance in veterinary entomology.*

The following locations have research projects addressing the issue identified under Problem Statement 3G:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas (some collaborative work was done in Laramie, Wyoming, before that location was closed in 2012).

Problem Statement 3G: Select Accomplishments

Blue-tongue virus detection techniques. Blue-tongue virus affects sheep and goats, but it also infects cattle. It is debilitating to animals because it makes their mouths extremely sore, disrupting feeding and sometimes killing the animals. The disease is commercially disruptive through direct damage to the animals and also because its occurrence stops shipment of animals from an area. A new form of blue-tongue virus spread through much of Europe recently, including areas that had never experienced blue-tongue of any kind. That outbreak also revealed the existence of a strain, BTV-8, that is pathogenic in cattle. Accurate detection of the virus is essential for the livestock industry because presence of exotic strains of the virus is a cause for quarantine. There is also a need to target resources for vector control. ARS scientists in Manhattan, Kansas, have developed new, rapid-detection methods that distinguish the specific strain of blue-tongue virus infecting an animal. One method uses infrared light to detect positive tests based on DNA amplification. The other detects DNA directly by hybridization on a solid phase substrate. The method was used to determine the strain of blue-tongue virus infecting deer

on cervid farms in Oklahoma. Broad application of this technique could map the presence of blue-tongue virus throughout the country, showing where control of the biting midge vectors was important. The work has helped the National Animal Health Laboratory Network document occurrence of blue-tongue virus in the United States.

Kato, C.Y. and Mayer, R.T. 2007. An improved, high-throughput method for detection of bluetongue virus RNA in *Culicoides* midges utilizing infrared-dye-labeled primers for reverse transcriptase PCR. Journal of Virological Methods 140(1), 140-147.

Effect of virus infection on biting midge feeding. Biting midges have come into greater prominence as veterinary vectors in recent years because of the wave of exotic blue-tongue virus infections and, in 2011, the appearance of Schmallenberg virus in Europe. Vesicular stomatitis virus continues to be a severe problem in tropical America, both harming animals and creating a constant need to determine whether or not an animal is infected with foot-and-mouth virus, since the signs for the two diseases are similar. ARS entomologists in Manhattan, Kansas, demonstrated that biting midges infected with vesicular stomatitis virus do not feed as successfully. The delay in feeding increases the likelihood that the virus will reach infective levels in more individual midges. Similar delays in feeding were observed in midges infected with blue-tongue virus. The researchers showed that the virus-induced delay in blood feeding gave the midge extra time to develop an infective level of viremia. This is significant because the risk of mortality for the midge is high each time it feeds, due to host defensive behavior. The virus-induced delay therefore increases the chance that an individual midge will live long enough to transmit the virus. Risk estimates of vesicular stomatitis and blue-tongue virus transmission would normally be based on longevity of the midge population taken as a whole. This discovery shows that longevity should be estimated based on the infected population, potentially causing a great change in estimates of risk.

Bennett, K.E., Hopper, J.E., Stuart, M.A., West, M.S., and Drolet, B.S. 2008. Blood-feeding behavior of vesicular stomatitis virus infected *Culicoides sonorensis* (Diptera: Ceratopogonidae). Journal of Medical Entomology 45: 921-926.

Studies on the saliva of biting midges. Pathogen infection from vectors is usually affected by salivary components of the vector. In some cases, the host's immune response to salivary components can be protective, whereas the saliva may actually enhance infection in hosts that have had less exposure to the bites of the particular vector. ARS researchers in Manhattan, Kansas, working on the components of biting midge saliva, showed that at least 55 proteins are present. These proteins have various effects on mammals, including vasodialation and anti-coagulation. One component in particular, a 66kDa protein with a maltase function, was shown to be the probable cause of summer eczema ("sweet itch") in horses. The researchers demonstrated this by comparing the effects of the native protein and the same protein generated from transformed, cloned bacteria. In addition to its significance in understanding biting midge biology, this research raises the possibility that salivary components could be important additives for vaccines.

Langner, K.F., Jarvis, D.L., Schuberth, H., Nimtz, M., Heselhaus, J.E., Mcholland, L.E., Leibold, W., and Drolet, B.S. 2009. Identification, expression, and characterization of a major salivary allergen (Cul s 1) of the biting midge *Culicoides sonorensis* relevant for summer eczema in horses. International Journal for Parasitology 39: 243-250.

Langner, K., Darpel, K.E., Drolet, B.S., Fischer, A., Hampel, S., Heselhaus, J.E., Mellor, P.S., Mertens, P.,and Leibold, W. 2008. Comparison of cellular and humoral immunoassays for the assessment of summer eczema in horses. Veterinary Immunology and Immunopathology 122: 126-137.

Potential control techniques for biting midges. Testing the efficacy of applications against biting midges is a challenge because of the difficulty of counting the insects on the host. Using tent enclosures and colony-reared biting midges, ARS scientists in Laramie, Wyoming, were able to use commercial products to prevent biting on sheep, a practical procedure to prevent exposure to blue-tongue virus as sheep return from mountain pastures in the western United States. Both PYthon ear tags with 10 percent zeta-cypermethrin (9.8 g per tag) synergized with 20 percent piperonyl butoxide (PBO), and a 12-ml low-volume spray application of ready-to-use sheep insecticide (Y-TEX) with 2.5 percent permethrin and 2.5 percent PBO in an oil-based formulation were repellent to *Culicoides sonorensis* for at least 3 to 5 weeks after a single application. The results of this work changed practices in the sheep industry and resulted in interest from the deer industry to develop methods to protect those animals. Efficacy testing of commercial products for control of pests that the manufacturer may have added to the label with little data is a very necessary part of agricultural research. Without such work, producers can only use the products based on the assumption that they will work, rather than based on evidence.

Reeves, W.K., Lloyd, J.E., Stobart, R., Stith, C.E., Miller, M.M., Bennett, K.E., and Johnson, G. 2010. Control of *Culicoides sonorensis* (Diptera: Ceratopogonidae) blood feeding on sheep with long lasting repellent pesticides. Journal of Economic Entomology 26: 302-305.

PROBLEM STATEMENT 3H**:** *Phlebotomine sand flies transmit leishmaniasis and sand fly fever virus; current methods for control are not uniformly effective. This group of biting flies is seldom collected in the United States, even though it is abundant even in northern locations. Livestock are completely exposed to the bites of phlebotomine sand flies with unknown health consequences.*

The following locations have research projects addressing the issue identified under Problem Statement 3H:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas (some collaborative work was done in Laramie, Wyoming, before that location was closed in 2012).

Problem Statement 3H: Select Accomplishment

Range expansion and associated species of Lutzomyia apache. Because of the documented association between vesicular stomatitis virus and phlebotomine sand flies in Georgia, ARS scientists in Laramie, Wyoming, performed surveys for sand flies in that State. Their work depicted an extension of the range of the sand fly *Lutzomyia apache* northward and higher in elevation, including into areas of Wyoming where transmission of vesicular stomatitis virus has

occurred. Thorough examination of the sand flies did not find vesicular stomatitis virus, *Bartonella*, or *Wolbachia* (a symbiont of many kinds of insects and potentially useful for control). However, a species of *Rickettsia*, an ascogregarine parasite, and parasitic mites were found. Although this study did not find pathogens of livestock, the fact that these inconspicuous blood-feeding insects are present in unexpected habitats raises the question of whether they might be important vectors of some pathogens. The results of this work have led ARS to continue sand fly research from a veterinary, as well as human, health standpoint.

Reeves, W. K., Kato, C. Y., and Gilchriest, T. 2008. Pathogen screening and bionomics of *Lutzomyia apache* (Diptera: Psychodidae) in Wyoming, USA. Journal of the American Mosquito Control Association 24(3), 444-447.

PROBLEM STATEMENT 3I**:** *Imported and established in the United States, invasive vectors of livestock pathogens could threaten the industry by transmitting new pathogens.*

The following locations have research projects addressing the issue identified under Problem Statement 3I:

- Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.

Problem Statement 3I: Select Accomplishment

Successful forecast of an outbreak of Rift Valley fever. To prevent potential introduction of Rift Valley fever virus into the United States, it is important to be able to detect and respond to outbreaks in natural settings in Africa and the Middle East. ARS scientists in Gainesville, Florida, have discovered a method that uses global and local climate and vegetation development information to forecast this disease well before it occurs. In late 2006, an early warning of an impending Rift Valley fever outbreak was issued to national and international agricultural and public health officials 3 months before a large outbreak occurred in four countries in the Horn of Africa.

This outbreak affected thousands of people and hundreds of thousands of domestic animals, dramatically impacting the economy of these countries. This was the first time that an

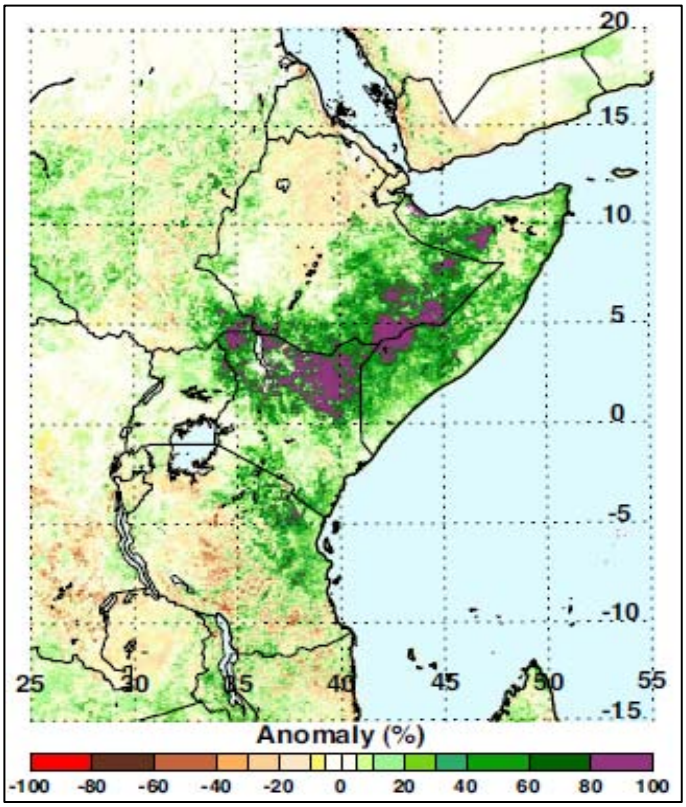

FIGURE 5: NDVI anomalies computed in December 2006 show percentage departures from the 1998-2006 mean. Positive anomalies are associated with above-normal rainfall and indicative of anomalous vegetation growth, creating ideal conditions for the emergence and survival of large populations of mosquito vectors. This information aided ARS scientists in predicting an impending Rift Valley fever outbreak.

alert resulted in increased national and international surveillance, enhancing the Rift Valley fever control response.

Anyamba, A., Chretien, J.P., Small, J., Tucker, C.J., Formenty, P.B., Richardson, J.H., Britch, S.C., Schnabel, D.C., Erickson, R.L., and Linthicum, K.J. 2009. Prediction of a Rift Valley fever outbreak. Proceedings of the National Academy of Sciences 106:955-959.

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COMPONENT 4: Pests that Damage Structures

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was introduced into the continental United States after World War II in infested materials shipped from the Pacific Far East, and it has since spread to 11 states. This particularly destructive termite can live in the ground or in nests it constructs within structures. The annual national cost for treatments and repair of the damage caused by the Formosan termite is estimated at $1 billion, excluding the value of trees lost to termite infestations. It is estimated that the population size of the termite in the New Orleans, Louisiana, area alone has expanded 35-fold in the previous decade. Other termites, such as the *Reticulitermes* native subterranean termite, also cause damage in areas where they occur.

ARS received funds for Formosan subterranean termite research for 15 years in response to a general threat to structures throughout the Southeast and a particular threat to the French Quarter of New Orleans. As with ARS' involvement in medical entomology, the Agency was selected for this work because of its entomological capacity. The program worked through the functional elements of IPM in cooperation with university and governmental partners.

ARS was the principle source of funding and research on termites in the United States until 2012, when the program was terminated. The timing coincided with budget cuts to the Agency; however, by this time, the program had developed an effective system for control of the Formosan subterranean termite. As pointed out in the accomplishments below, ARS can be credited with literally saving the historic French Quarter of New Orleans. That work required years of extensive collaboration and product development—a good example of ARS' ability to solve problems through research and development. The success of the program was the result of a closely coordinated cooperative funding program in which partners worked toward a common goal under ARS guidance and an in-house research program on biology and control of termites. One of the last achievements of the program was extensive description and annotation of the termite genome, an accomplishment that could lead to additional developments in the control of termites by other entities.

PROBLEM STATEMENT 4A: *Following the development of effective tools, the challenge to controlling Formosan subterranean termite is organization, creative technique, and application.*

The following locations had research projects addressing the issue identified under Problem Statement 4A:

- Formosan Subterranean Termite Research Unit, Southern Regional Research Center, New Orleans, Louisiana.
- Formosan Subterranean Termite Cooperative Research Program, Southern Regional Research Center, New Orleans, Louisiana.
- Natural Products Utilization Research Unit, Oxford, Mississippi.

Problem Statement 4A: Select Accomplishments

Discovery of new chemicals for termite control. Insecticides for termite control need to serve many purposes in terms of safety, effectiveness, longevity, and acceptability. Natural products have appeal because they are perceived as safe for residents and the environment. ARS scientists in Oxford, Mississippi, and New Orleans, Louisiana, discovered a naturally occurring chromene compound extracted from plants that kills termites. ARS was awarded a patent for the compound, which led to exploration of analogous compounds that were even more effective. In addition, a fungal extract containing a single chemical that acted as a powerful attractant and feeding stimulant to the termites was found. This work may lead to completely new termiticides with low mammalian toxicity. The attractant could be combined with toxicants to make more effective termite control devices.

In complementary research, ARS scientists developed a carbohydrate-degrading enzyme inhibitor that is potent, stable, non-toxic, and biodegradable. Bioassays showed that there is no feeding repellency or rejection with choice tests at lethal concentrations. Lethal effects occurred 5 to 6 days after voluntary feeding of the inhibitor-treated cellulosic materials, resulting in 91 to 100 percent mortality after another 5 to 8 days.

Nakayama, F.S. and Osbrink, W.L. 2010. Evaluation of kukui oil (*Aleurites moluccana*) for controlling termites. Industrial Crops and Products 31 (2): 312-315.

Hussain, A., Tian, M., He, Y., Bland, J.M., and Gu, W. 2010. Behavioral and electrophysiological responses of *Coptotermes formosanus* Shiraki towards entomopathogenic fungal volatiles. Biological Control 55:166-173.

Cornelius, M.L., and Osbrink, W.L. 2009. Bioassay design and length of time in the laboratory affect intercolonial interactions of the Formosan subterranean termite (Isoptera, Rhinotermitidae). Insectes Sociaux 56 (2), 203-211.

Areawide integrated management of the Formosan subterranean termite. ARS scientists in New Orleans, Louisiana, conducted research on new ways to control this termite and on forming a strategy for community control. Although the termite continues to spread widely in the South, this program conclusively demonstrated between 1997 and 2011 that an IPM program against the termite can be very effective. The demonstration project in New Orleans achieved 95 percent control of the termite, as measured by the disappearance of colonies from individual buildings and public spaces. The techniques used to achieve this level of control were based on a wide range of studies, including how termites develop to specialized forms, identification of individual colonies by their genetic signatures, invention of new kinds of monitoring and bait stations, detection equipment based on sound and infrared, and novel methods of treating living trees. In the course of this project, new classes of insecticide were developed, the complete genome of the termite was sequenced, and novel enzymatic pathways for energy production were discovered. The efforts of ARS research on termites can be credited with most of the development of modern termite control in the United States, which protects structures effectively without the negative environmental consequences of older treatment methods.

Guillot, F.S., Lax, A.R., Ring, D.R., Morgan, A., Brown, K., Riegel, C., and Boykin, D.L. 2010. Area-wide management of the Formosan subterranean termite, *Coptotermes formosanus,* Shiraki (Isoptera: Rhinotermitidae) in the New Orleans French Quarter. Sociobiology 55 (2): 311-338.

Lax, A.R. and Wiltz, B.A. 2010. Swarming of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in southern Mississippi. Midsouth Entomologist 3 (1):18-25.

Wiltz, B.A., Woodson, W.D., and Puterka, G.J. 2010. Potential of kaolin-based particle film barriers for Formosan subterranean termite (Isoptera: Rhinotermitidae) control. Sociobiology 55 (2):405-413.

Cornelius, M.L. 2008. Changes in the distribution of subterranean termite colonies (Isoptera: Rhinotermitidae) in City Park, New Orleans, Louisiana, in the aftermath of hurricane Katrina. Proceedings of the 6th International Conference on Urban Pests pp. 455-460. http://www.icup.org.uk/reports/ICUP919.pdf

Cornelius, M.L., Duplessis, L.M., and Osbrink, W.L. 2007. The Impact of hurricane Katrina on the distribution of subterranean termite colonies (Isoptera: Rhinotermitidae) in City Park, New Orleans, Louisiana. Sociobiology 50 (2):1-25.

PROBLEM STATEMENT 4B**:** *Invasive and native termites are a threat to structures, which has led to greater damage than any other group of pests in the United States.*

The following location has research projects addressing the issue identified under Problem Statement 4B:

- Formosan Subterranean Termite Research Unit, Southern Regional Research Center, New Orleans, Louisiana.

Problem Statement 4B: Select Accomplishments

Biochemistry and genetics of the Formosan subterranean termite. Genetic description of termites offers the hope of finding completely new ways to control these pests. The Formosan subterranean termite and other invasive and native termites are the most expensive pests in the United States, so any refinement in their control is important. The great expense from termites is a result of the damage they do to structures and the considerable investment to prevent termite infestation. ARS scientists in New Orleans, Louisiana, in collaboration with the J. Craig Venter Institute and Purdue University, compared the genome of the Formosan subterranean termite with the native subterranean termite (*Reticulitermes flavipes*). Approximately 80x coverage of the 926 mb genomes was completed using Illumina Nextgen sequencing during this reporting period. As part of the description of the genome, 11 of 15 genes belonging to the metabolic mevalonate pathway were identified. The researchers developed phenotypic and gene expression profiles for these genes. This pathway is responsible for the production of juvenile hormone, which controls many aspects of termite development and caste specialization. Phenotypic bioassays with chemicals that promote and inhibit the mevalonate pathway provided further information on the physiology of this essential aspect of termite biochemistry. Chemicals that up-regulate the pathway increased worker-to-soldier differentiation, whereas chemicals that inhibited the pathway decreased the worker-to-soldier differentiation.

The genetic research on the Formosan subterranean termite also produced gene-protein sequences and gene-protein expression profiles of the myosin heavy chain, contrasting worker and soldier castes, as well as between body regions of termites.

ARS scientists in New Orleans, Louisiana, cloned two enzymes from the Formosan subterranean termite and expressed them in a bacterial expression system. Incubation of the two enzymes together with cellulose results in digestion of the cellulose to glucose. This will allow testing of a variety of possible inhibitory compounds against the digestive enzymes necessary for the termites, with possible use as a wood preservative or termite control agent. An additional outcome for this research is the possible use of these enzymes for the conversion of biomass into biofuel.

In separate biochemical characterization, the scientists identified and characterized a novel endogenous endo-ß-1,4-glucanase (named CfEG5) in the Formosan subterranean termite. They also described the gene structure, expression, function, specificity, kinetics, and phylogenetic relatedness of CfEG5.

Tarver, M.R., Mattison, C.P., Florane, C.B., Hinchliffe, D.J., Zhang, D., and Lax, A.R. 2012. Screening of multiple potential control genes for use in caste and body region comparisons using RT-qPCR in *Coptotermes formosanus*. Sociobiology 59 (1):81-96.

Zhang, D., Allen, A.B., and Lax, A.R. 2012. Functional analyses of the digestive β-glucosidase of Formosan subterranean termites (*Coptotermes formosanus*). Journal of Insect Physiology 58 (1):205-210. http://dx.doi.org/10.1016/j.jinsphys.2011.11.014.

Zhang, D., Lax, A.R., and Bland, J.M. 2011. Characterization of a new endogenous endo-β-1,4-glucanase of Formosan subterranean termite (*Coptotermes formosanus*). Journal of Insect Biochemistry and Molecular Biology 41:211-218.

Zhang, D., Lax, A.R., Raina, A.K., and Bland, J.M. 2009. Differential cellulolytic activity of native-form and C-terminal tagged-form cellulase derived from *Coptotermes formosanus* and expressed in E. coli. Insect Biochemistry and Molecular Biology 39:516-522

Bland, J.M., Raina, A.K., Carpita, A., and Dickens, J.C. 2007. Comparative analysis of 3,6,8-dodecatrien-1-ol from three subterranean termite species. Sociobiology 50 (2):535-531.

Origin of the invasive Formosan subterranean termite. The dynamics of invasive species depend greatly on the genotypes introduced into their new ranges. Continuous introduction of new genetic pools creates a more difficult problem than if there were only one or a few introductions with limited genetic variability. ARS scientists in New Orleans, Louisiana, collected 28 Formosan subterranean termite colonies from 2004 to 2008, including 21 colonies from Mississippi, six colonies from Louisiana, and one from China. Two genotypes of Formosan subterranean termites were identified in Mississippi, where the first one, GA type, was identical to those reported previously in Georgia, Louisiana, Alabama, and other infested states. The second, AT type, was identified for the first time in the southeastern United States. Sequence identity of the AT type of Formosan subterranean termite with those reported mainly in China provided evidence of at least two introductions of the termite to the United States. The termites in Mississippi are potentially transported from the State's port cities along the Gulf of Mexico. Numerous infestations have been observed along transportation corridors via Interstate

59 in Mississippi and Highway 11, as well as a parallel railway from New Orleans, Louisiana, to Meridian (Lauderdale County), Mississippi. The genetic diversity of the Formosan subterranean termite in Mississippi was expected to be higher than in other inland states. However, besides a recently introduced colony from New Orleans with AT type, 20 colonies collected in south Mississippi had identical GA genotype. Understanding that only two introductions of the termite have occurred gives rise in confidence to the use of control techniques that target specific gene sequences. This knowledge also enables the detection of additional introductions and when they happened.

Yang, C., Shoemaker, D.D., Wu, W., and Shih, C. 2008. Population genetic structure of the red imported fire ant, *Solenopsis invicta*, in Taiwan. Insectes Sociaux 55:54-65.

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COMPONENT 5: Fire Ants, Other Invasive Ants, and Household Pests

Imported fire ants inhabit over 350 million acres in 12 southern States, from Texas to Virginia, and Puerto Rico, and they have recently become established in limited areas in California and New Mexico. U.S. economic and medical costs of fire ants exceed $6 billion dollars annually, and commercial chemical treatments for control are estimated to cost as much as $40 per acre. Chemical control is effective in limited geographic areas, but it is costly and provides only temporary fire ant suppression. Other ants, particularly imported pests, are also problematic. The risk that a damaging invasive ant will become established is greatly influenced by its interactions with native species of ants.

NP 104 has historically conducted research on purely household pests, such as cockroaches. However, the current direction of this Component is concentrated principally on fire ants (the red imported fire ant and somewhat less on the black imported fire ant), although attention has been expanded to other ants, especially those that threaten to behave as invasive species. Ants are essential to agriculture as providers of ecological services in the soil, but they are also severe pests at times on certain crops and livestock through direct damage and by protecting homopterous pests from predators and parasitoids.

The program works closely with APHIS and local governments on production and distribution of biological control agents against fire ants. The researchers in this National Program have formed important partnerships with industry for the improvement of fire ant baits, repellents against fire ants, and new toxicants to control fire ants.

The accomplishments for Component 5 are aligned under the four Problem Statements as noted in the Action Plan.

PROBLEM STATEMENT 5A**:** *Imported fire ants threaten the safety of people on their property, the well-being of farm animals, and the condition of pastures.*

The following locations have research projects addressing the issue identified under Problem Statement 5A:

- Imported Fire Ant and Household Insect Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Biological Control of Pests Research Unit, National Biological Control Laboratory, Stoneville, Mississippi.

Problem Statement 5A: Select Accomplishments

Remote sensing of fire ant mounds. The distribution of any insect is an important piece of information used to target control efforts. The imported fire ant makes mounds of dirt over its nests, which are the best places to put toxic baits. ARS scientists in Stoneville, Mississippi, have found that the total volume of fire ant mounds is the same whether colonies are of the multiple queen or single queen type. The mounds can be detected from aerial photography by measuring

five distinct wavebands in the visible, near-infrared, and mid-infrared. Detection and quantification of fire ant mounds from the air could provide an important tool for evaluation of areawide control efforts, including the successful establishment of biological control agents discovered by ARS. On a smaller scale, detection of mounds within a field or pasture could help target the use of pesticide baits.

Defauw, S.L., Vogt, J.T., English, P.J., and Boykin, D.L. 2009. Seasonal Dynamics of Hyperspectral Reflectance Patterns Influencing Detection of Imported Fire Ant (Hymenoptera: Formicidae) Mound Features in Turfgrass. Journal of Entomological Science 44:287-294.

Defauw, S.L., Vogt, J.T., and Boykin, D.L. 2008. Influence of mound construction by red and hybrid imported fire ants on soil chemical properties and turfgrass in a sod production agroecosystem. Insectes Sociaux

Vogt, J.T., Wallet, B., and Freeland, T.B. 2008. Imported fire ant (Hymenoptera: Formicidae) mound shape characteristics along a north-south gradient. Environmental Entomology 37:198-205.

Functional genomic studies of fire ants. Genomic studies provide insights into many aspects of physiology, behavior, and population dynamics. ARS scientists in Gainesville, Florida, played a leadership role in organizing a consortium of researchers to sequence the genome of the fire ant *Solenopsis invicta*. A complete draft genome of the fire ant was generated, resulting in a resource for future evolutionary and functional genomics studies on this pest. Several unique aspects of the fire ant genome were described that are likely linked to the complex social behavior of this species, including odorant reception and tolerance of a colony for multiple queens (polygyny). A subsequent study identified and enumerated the odorant-binding proteins (OBPs) from a single fire ant species. A total of 18 OBPs were identified, which is the smallest OBP repertoire found in an insect to date. The results of this OBP study underscore the importance of carefully exploring sensitivity to the effects of alignment methods for data comprising widely divergent sequences. Another study characterized the first ant circadian clock, demonstrating that two social insects—ants and bees—share a similar, mammalian-like circadian clock. This study represented the first characterization of clock genes in an ant and is a key step towards understanding socially regulated plasticity in circadian rhythms.

Additional genomic resources were developed for *S. invicta* by ARS scientists in Gainesville, Florida, including a high-density oligo-microarray that was utilized for several comparative gene expression studies in fire ants. The first study investigated gene expression patterns in different castes and life stages of two fire ant species to obtain a comprehensive picture of the relative contributions of gender (sex), caste, developmental stage, and species divergence to gene expression evolution. The results suggested much of the evolution of gene expression in fire ants may occur in the worker caste, despite the fact that these individuals are largely or completely sterile. Analyses of gene expression evolution also revealed a combination of positive selection and relaxation of stabilizing selection as important factors driving the evolution of such genes. A second study examined levels of gene expression in each of the three castes in North American populations of two introduced fire ants and in their hybrids. The results of this study indicated that only modest hybrid incompatibilities in gene expression exist between the two species. Nonetheless, significant heterogeneity in the degree of hybrid incompatibilities occurs across life

stages and castes, suggesting that each caste may contribute differently to the divergence and maintenance of separated species in these ants. Finally, a study that combined data on gene expression patterns and evolutionary rates of genes associated with castes, sexes, and developmental stages in fire ants demonstrated that (1) genes with diminished selective constraints have a higher propensity for evolving conditional expression associated with phenotypic plasticity, (2) the rate of molecular evolution is higher for conditionally expressed genes than those with ubiquitous expression, and (3) relaxed selection appears to precede differential gene expression. These combined results suggest that relaxed selection likely has played an underappreciated role in the evolution of phenotypic plasticity.

This work will provide the foundation for entirely novel methods to manage fire ant populations. An understanding of OBPs will make it possible to target behavior-altering chemicals *in silico* by matching structures to binding sites. Differential gene expression and variation in phenotype influence the capabilities of fire ant populations, knowledge of which will inform decisions on risk assessment.

Gotzek, D., Robertson, H.M., Wurm, Y., and Shoemaker, D.D. 2011. Odorant binding proteins (OBPs) of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): An example of the problems facing the analysis of widely divergent proteins. Molecular Biology and Evolution 6:1-9.

Wurm, Y., Wang, J., Riba-Grognuz, O., Corona, M., Nygaard, S., Hunt, B.G., Ingram, K.K., Falquet, L., Nipitwattanaphon, M., Gotzek, D., Dijkstra, M.B., Oettler, J., Shih, C., Wu, W., Scotty Yang, C., Thomas, J., Beaudoing, E., Pradervand, S., Flegel, V., Fabbretti, R., Stockinger, H., Long, L., Farmerie, B., Oakey, J., Harkins, T., Boomsma, J.J., Pamilo, P., Yi, S.V., Heinze, J., Goodisman, M.A., Farinelli, L., Harshman, K., Hulo, N., Cerutti, L., Xenarios, I., Shoemaker, D.D., and Keller, L. 2011. The genome of the fire ant *Solenopsis invicta*. Proceedings of the National Academy of Sciences 10:1073.

Ometto, L., Shoemaker, D.D., Ross, K.G., and Keller, L. 2010. Evolution of gene expression in fire ants: the effects of developmental stage, caste, and species. Molecular Biology and Evolution 28:1381-1392.

Evolutionary genomic studies of fire ants. Imported fire ants are prime examples of invasive species that, once located in a previously unoccupied range, may shuffle their genetic makeup to become pests with qualities previously unsuspected in their native range. Determination of the number of founders responsible for the establishment of invasive plant and animal populations is important for developing biologically based management practices, predicting the invasive potential of species, and making inferences about basic ecological and evolutionary processes. ARS scientists in Gainesville, Florida, developed numerous genetic markers to employ for population genetic studies of fire ants in their native and introduced ranges. A study to infer the makeup of the original founder population of fire ants in the United States was conducted utilizing genetic markers. Genotypic data from different classes of markers suggested that between 9 and 20 mated fire ant queens likely comprised the initial founder group that colonized Mobile, Alabama, in the mid-1930s. These results suggested that the rapid spread and massive population buildup of introduced fire ants occurred despite loss of substantial genetic variation associated with the relatively small number of founders. Among other purposes, these results can be used to reconstruct likely scenarios for the introduction of this and other ants with similar habits into other parts of the world, as well as to help provide a general foundation for

understanding how the loss of genetic diversity often associated with founder events may influence the evolution of invasive populations.

These extensive genetic markers also were utilized for delimiting species boundaries within a widespread problematic ant taxon. This study was aimed at distinguishing evolutionarily independent lineages within a widespread and highly variable fire ant species, *Solenopsis saevissima*. The integrated approach using diverse data types and methods of analysis revealed a range in levels of divergence and evolutionarily distinctiveness among regional populations of this single nominal fire ant species. The results illustrate the importance of employing different classes of genetic data, different methods of genetic data analysis, and different sources of data to provide robust delimitation of species boundaries in groups of recently diverged lineages such as fire ants. The global invasion history of fire ants was reconstructed using the genetic markers.

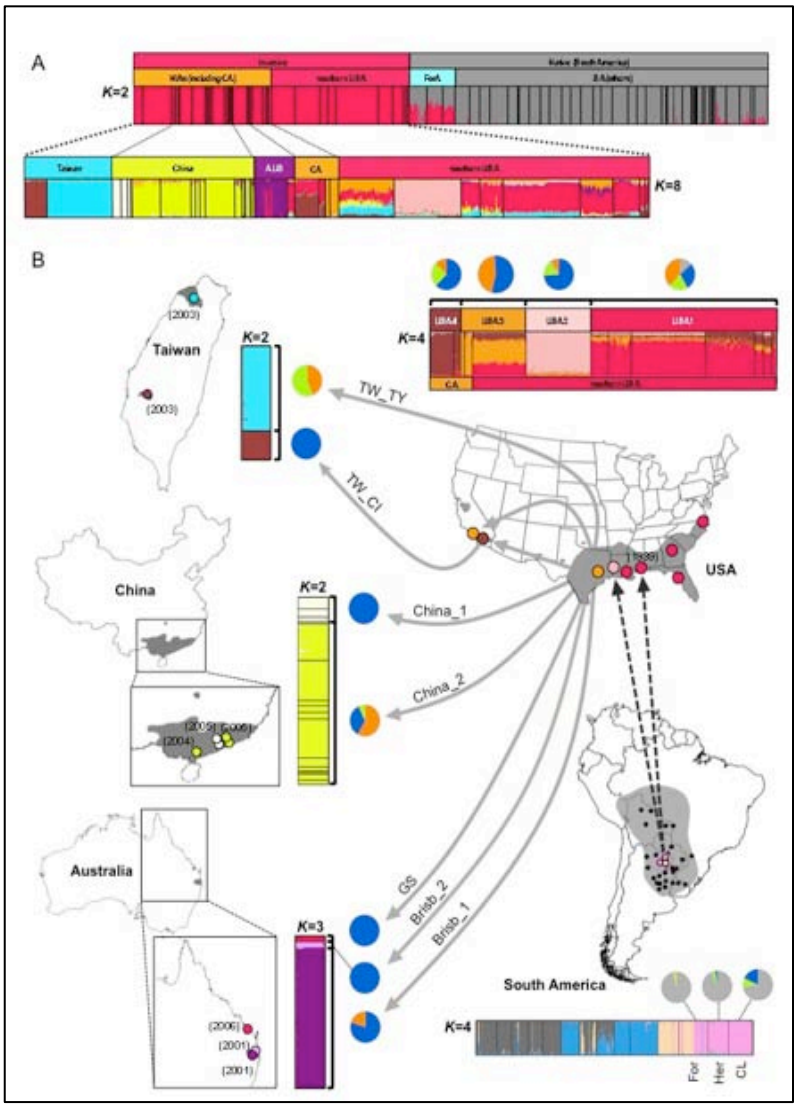

FIGURE 6: The graphic shows the geographic distribution of genetic types of red imported fire ants and diagrammatic representation (A) of marker distribution. Also shown is the calculated historical movement (B) of red imported fire ant genetic types from South America to North America, and subsequently to other countries.

Knowing the source of invasive fire ant infestations in the United States and other countries (e.g., Australia, China, and Taiwan) is important for guiding the search for biological control agents and for determining whether biocontrol agents used in the United States would be effective in other countries with this invasive ant. The results of this study showed that the imported red fire ant was genetically filtered in the United States before subsequent, accidental movement of populations to other countries. It is likely that *S. invicta* will continue to expand its range to other areas of the world due to increasing and interconnected global trade and travel system. Importantly, these results also suggest that biologically based fire ant control methods developed by ARS may be transferred to invasive fire ant populations in the other countries in this study.

Finally, a population genetic study was completed aimed at determining how commonly, if ever, fire ant queens mate with more than one male. Understanding fire ant male reproductive success and fitness are important components of research aimed at suppressing fire ant populations, yet data on this important topic were virtually nonexistent. This study revealed that some fire ant queens mate with more than one male and, unexpectedly, that whether a queen mates with more than a single male is determined almost entirely by male genotype. Investigation of the physiological basis for the inability of some males to discourage a second mating revealed that male sperm count also is linked to male genotype, suggesting that fire ant queens remain receptive to mating if their first partner does not provide a sufficient quantity of sperm. This study provided a first view of the fitness of male fire ants and established how deleterious variation with respect to male fitness can be maintained as a consequence of being linked to genes harboring adaptive variation controlling social organization. This basic discovery has implications for management of fire ant species, especially for estimates of the speed with which new traits might spread into a population.

Ascunce, M.S., Yang, C., Oakey, J., Calcaterra, L., Wu, W., Shih, C., Goudet, J., Ross, K.G., and Shoemaker, D.D. 2011. Global invasion history of the fire ant *Solenopsis invicta*. Science 331:1066-1068.

Ross, K.G., Gotzek, D., Ascunce, M.S., and Shoemaker, D.D. 2010. Species delimitation: A case study in a problematic ant taxon. Systematic Biology 59(2): 162-184. doi:10.1093/sysbio/syp089

Ross, K.G. and Shoemaker, D.D. 2008. Estimation of the number of founders of an invasive pest insect population: the fire ant *Solenopsis incivta* in the United States. Proceedings of the Royal Society of London B.2231-2240.

The black imported fire ant. The preponderance of research on the genetics of the imported fire ant has focused on red imported fire ants. With expansion of the hybrid imported fire ant, *Solenopsis invicta x richteri*, into more northerly regions, there has been a need for a better understanding of any difference in characteristics of this form compared to either of the two pure species. ARS scientists in Gainesville, Florida, have expanded the application of mitochondrial DNA markers using a technique first performed with the red imported fire ant. Testing imported fire ants from eight states identified five distinct haplotypes in red imported fire ants and a single haplotype in black imported fire ants. All but one of the rarer red ant types has been detected in the hybrid ants thus far. The single black ant haplotype is also present in hybrid populations. This test adds to other markers that aid in species identification and population metrics. Given that the use of morphological characters alone for identification in this group is problematic, additional genomic tools are a useful addition. There is evidence of multiple inseminations in the hybrid imported fire ant, shown by the examination of sperm genotypes of newly mated fire ant queens. This determination runs contrary to what has been reported in red imported fire ant literature.

Fritz, G.N., Fritz, A.H., and Vander Meer, R.K. 2011. Sampling high-altitude and stratified mating flights of red imported fire ant. Journal of Medical Entomology 48(3):508-512.

Viruses for control of fire ants. Biological control depends on establishing a complex of natural enemies that sustainably impact the target insect so that average population levels are below the economic threshold. Biological control agents introduced to date against fire ants have only

partly accomplished this goal. The extensive genomic studies above, including sequencing and annotation of the complete genome, were utilized to find viruses that are pathogenic to fire ants. These viruses were the first of any kind found in ants and included a unique kind of RNA virus, SINV-3, that is particularly lethal to fire ants. By comparing SINV-3 from American and Argentinean populations, ARS scientists in Gainesville, Florida, and Hurlingham, Buenos Aires, in Argentina, found that the genomic architecture was different for the two populations. Further development of these viruses may produce a different kind of biological control agent capable of reducing populations of fire ants in the United States.

Valles, S.M., Allen, C., Varone, L., and Briano, J. 2010. Complete genome sequence of an Argentinean isolate of *Solenopsis invicta* virus 3. Virus Genes 40:293-297.

Valles, S.M. and Hashimoto, Y. 2008. Characterization of structure proteins of *Solenopsis invicta* virus 1 virus research. Virus Research 136(1):189-191.

Valles, S.M. and Porter, S.D. 2007. Pseudacteon decapitating flies: potential vectors of a fire ant virus? Florida Entomologist 90(1):268-270.

RNAi as an insecticide against fire ants. Pesticides for fire ants often have non-target effects against other insects and other ants. Genomic data on fire ants were used by ARS scientists in Gainesville, Florida, to aid in identifying putative sequences for the PBAN/Pyrokinin (produces neuropeptide ligands) and PBAN receptor (responds to ligands) genes. The PBAN/Pyrokinin and PBAN receptor genes were characterized in the fire ant, and double-stranded RNA (RNAi) constructs for both were shown to have deleterious effects on fire ant colonies. The potential utility of the RNAi methodology for this gene was expanded successfully to a pest moth species, and specific RNAi constructs are likely to have utility against a broad range of insect pests. Several patents have been filed to protect this novel control method, and there is commercial interest.

ARS scientists in Stoneville, Mississippi used a similar RNAi approach to reduce expression of a G-protein gene involved in the functioning of critical G-protein coupled receptors. A single feeding of these preparations was sufficient to eliminate a colony in the laboratory. A patent has been filed to protect this novel control method, and there is commercial interest.

The two above examples of RNAi in fire ants represent the first proof of concept for the utility of RNAi to control pest ants, a concept developed by ARS. Such a method would be highly specific for fire ants, leveraging the efforts of native species of ants to compete with the invasive fire ants. Combined with established methods of biological control (parasites and pathogens that kill fire ants), these new methods will contribute toward restoring ecological balance where fire ants currently reach an abundance not experienced in their native range. Such success in biological and biologically based control would reduce the use of insecticides and improve crop productivity and animal health and lessen stress caused by fire ants in many other economic sectors in the Southeast.

Vander Meer, R.K. and Choi, M.Y. 2011. Pheromone biosynthesis activating neuropeptide (pban)/pyrokinin family of peptides and fire ants, *Solenopsis* spp. Formosan Entomologist 31:133-147.

New toxicants for use against fire ants. The number of toxicants appropriate for use against fire ants is limited by effectiveness and environmental safety concerns. ARS researchers in Stoneville, Mississippi, have identified defensive chemicals in the Caribbean crazy ant (*Nylanderia* sp. near *pubens*), a new exotic invasive pest ant species first found near Houston, Texas, in 2002, and established a cDNA library for this species. This research is important for the use of ant-based defensive chemicals in managing imported fire ants and in understanding the biosynthesis pathways of these chemicals. In separate efforts, ARS researchers are screening natural products by exposing model colonies to plant or fungal extracts and observing the total effect on colony survival and behavior. A spin-off of this research was the discovery that fire ant cuticular hydrocarbons change composition seasonally.

The discovery of the first neuropeptide in fire ants may lead to additional physiological targets for chemical control. The function of the neuropeptide PBAN (pheromone biosynthesis activating neuropeptide) may be involved in alarm pheromone production. ARS researchers in Gainesville, Florida, studied the accumulation of a pyrazine alarm pheromone component from female sexual alate to newly mated queen and through the development of a mature colony. Periods of slow, rapid, and no accumulation were discovered. These data will be used to determine whether pheromone accumulation can be manipulated using PBAN or antagonists of the PBAN receptor. Interference of this peptide or the gene that produces it could lead to novel, non-insecticide methods for fire ant control.

Choi, M.Y., Vander Meer, R.K., Shoemaker, D.D., and Valles, S.M. 2011. PBAN gene architecture and expression in the fire ant, *Solenopsis invicta*. Journal of Insect Physiology 57(1):161-165.

Chen, J., Shang, H., and Jin, X. 2010. Interspecific variation of delta-1,6-piperideines in imported fire ants. Toxicon 55(2010)1181-1187.

Chen, J. 2009. Repellency of an over-the-counter essential oil product from China against workers of red imported fire ants. Journal of Agriculture and Food Chemistry 57(2):618-622.

Biological control of the fire ant. Biological control is the best hope for a sustainable solution to the fire ant problem in the United States and other countries where fire ants are an invasive species. Successful introduction of biological control agents have reduced the problem, but fire ants remain a hazard for people and domestic animals. ARS researchers in Gainesville, Florida, and Hurlingham, Buenos Aires, in Argentina, have been working together for more than 20 years to discover and develop biological control agents against the red

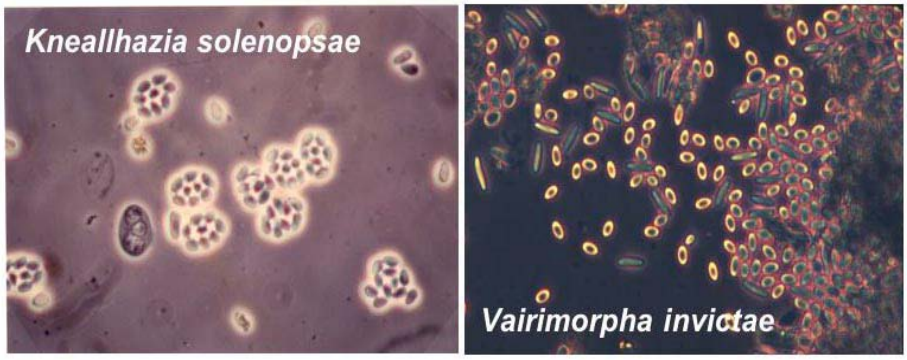

FIGURE 7: Two microsporidian parasites from South America (*Kneallhazia solenopsae* and *Vairimorpha invictae*) are being developed for use as biological controls of imported fire ants.

and black imported fire ants. Exploration in South America resulted in collection of a group of phorid flies and two microsporidian parasites (*Kneallhazia solenopsae* and *Vairimorpha invictae*). In addition, the scientists discovered three viruses and characterized them in the fire ant.

A cooperative study conducted with APHIS and State cooperators in 11 states documented that the first two species of fire ant-decapitating flies (*Pseudacteon tricuspis* and *Pseudacteon curvatus*) have expanded across almost the entire southeastern United States and Puerto Rico. Two biotypes of the second species (*P. curvatus*) were released, one on black and hybrid imported fire ants and the other on red imported fire ants. ARS scientists showed that densities of *P. curvatus* are about 10 times those of the first species (*P. tricuspis*). Other studies document the establishment and slow expansion of a third species (*P. litoralis*) at a site in Alabama and the rapid expansion of a fourth species (*P. obtusus*) across North Central Florida, where it is competing very well with the more abundant *P. curvatus*. APHIS and its State cooperators are currently releasing *P. obtusus* at sites across the range of red imported fire ants in the United States.

FIGURE 8: The phorid fly, *Pseudaction tricuspis*, about to insert an egg in a red imported fire ant worker.

ARS researchers have obtained approval from the North American Plant Protection Organization and APHIS regulators to release a fifth species of phorid decapitating fly (*Pseudacteon cultellatus*) as a fire ant biocontrol agent. This new species of fly specializes in attacking the smallest sizes of fire ant workers, which are most abundant in multiple-queen fire ant colonies. This preference is especially important because multiple-queen fire ant populations are larger and more difficult to control than single-queen fire ant populations. Thus, they are a substantially greater pest of homes, agriculture, and the environment. This species have now been established at several sites in Florida, were populations are being monitored.

ARS scientists in Gainesville, Florida, are currently conducting a regional sampling to compare historical and current fire ant densities along roadsides before and after the release of the fire ant-decapitating fly species and the discovery of fire ant viruses and microsporidian pathogens. Preliminary results show reductions in fire ant populations in some areas where fire ants were formerly abundant.

The researchers have also confirmed that *Kneallhazia solenopsae* infects *Solenopsis geminata,* a native North American ant species that is a pest in Hawaii and elsewhere. These results indicate that this pathogen has a broader host range than previously reported. This parasite was never intentionally introduced, but it may have come to the United States with imported fire ants brought into the country accidentally. Two distinct molecular variants of the parasite were found. One variant was widely distributed (South America, the United States, and southern Mexico), whereas the other variant was detected only in northern Mexico and Texas. The discovery of two variants suggests the potential for identifying more effective strains of fire ant

pathogens that can be used for biological control. It was also shown that phorid parasitoids of fire ants acquire *K. solenopsae* if they develop in infected fire ants. However, there is no evidence that the flies are vectors of the pathogen.

This research, and its implementation by APHIS, has had an effect on fire ant populations, with no danger of chemical contamination or occupational hazard. The research continues toward a time when introduced natural enemies will reduce fire ants from a major to a minor problem in the United States.

Ascunce, M.S., Yang, C., Oakey, J., Calcaterra, L., Wu, W., Shih, C., Goudet, J., Ross, K.G., and Shoemaker, D.D. 2011. Global invasion history of the fire ant *Solenopsis invicta*. Science 331:1066-1068.

Callcott, A., Porter, S.D., Weeks, R.D., Graham, L.C., Johnson, S.J., and Gilbert, L.E. 2011. Fire ant decapitating fly cooperative release programs (1994-2008): Two *Pseudacteon* species (*P. tricuspis*, *P. curvatus*) rapidly expand across imported fire and populations in the southeastern United States. Journal of Insect Science 2:1-25.

Porter, S.D., Graham, L., Johnson, S.J., Thead, L.G., and Briano, J.A. 2011. The large decapitating fly *Pseudacteon litoralis* (Diptera: Phoridae): Successfully established on fire ant populations in Alabama. Florida Entomologist 94:208-213.

Oi, D.H., Valles, S.M., and Briano, J. 2010. Laboratory host specificity testing of the fire ant microsporidian pathogen *Vairimorpha invictae* (Microsporidia: Burenellidae). Biological Control 53:331-336.

Valles, S.M. and Hashimoto, Y. 2009. Isolation and characterization of *Solenopsis invicta* virus 3, a new positive-strand RNA virus infecting the red imported fire ant, *Solenopsis invicta*. Virology 388:354-361.

PROBLEM STATEMENT 5B**:** *Other invasive ants in the United States (e.g., Argentine ant, little fire ant, crazy ants, the tropical fire ant, white-footed ant, and carpenter ants) are either difficult to control or threaten to become established pests in the United States.*

The following locations have research projects addressing the issue identified under Problem Statement 5B:

- Imported Fire Ant and Household Insect Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Biological Control of Pests Research Unit, National Biological Control Laboratory, Stoneville, Mississippi.

Problem Statement 5B: Select Accomplishments

Ant ecology related to fire ant infestations. Ants are conspicuously important to the environment, especially as native complexes of species. Introduction of some invasive ants has disrupted the ecological services provided by native ants. Preliminary surveys of ants by ARS cooperators at Mississippi State University in selected Mississippi nurseries that obtained containerized plants from Florida resulted in new distributional records within the State of several exotic ant species and one new State record for an invasive species, *Tapinoma*

melanocephalum (the ghost ant). A survey of ants in the Noxubee National Wildlife Refuge was also completed, resulting in a total of 96 species, including 8 new State records, 8 exotic species, and 2 possibly new and undescribed species. A cooperative project with the Natchez Trace National Parkway was conducted by ARS scientists in Stoneville, Mississippi, to measure changes in the ant community in prairie remnants where eastern red cedar is being removed by different methods. The range of the hybrid imported fire ant, *Solenopsis invicta* x *richteri*, was found to be expanding, especially where the black imported fire ant occurs. Because polygyny (a colony with multiple queens), and the higher population density associated with this form, is also being introduced with the black imported fire ant populations, a greater impact will be felt as the black imported fire ant is supplanted by the hybrid. Developing a picture of the current status of native and invasive ant species will be essential for determining whether or not control programs, especially biological control programs, are effective in restoring the ecological services provided by native ants.

Gardner, W.A., Diffie, S., Vander Meer, R.K., and Brinkman, M.A. 2008. Distribution of the fire ant (Hymenoptera: Formicidae) hybrid in Georgia. Journal of Entomological Science 43(1):133-137.

Progress toward control of the Caribbean crazy ant. The Caribbean crazy ant, *Nylanderia fulva* (formerly *Paratrechina* or *Nylanderia pubens*), appeared in the 1950s in the Southeast and became a locally severe problem in the late 1990s. A Texas pest control professional, Tom Rasberry, worked with the ants, leading to its earlier common name, the Rasberry crazy ant. Subsequent taxonomic study showed that it was the same as the Caribbean crazy ant. For unknown reasons, the ant has changed from being simply an introduced species to an invasive one, as it grows to huge populations in some areas. ARS scientists at Gainesville, Florida, have initiated studies to develop long-lasting, effective approaches to control the Caribbean crazy ant. Using a two-pronged approach of applied and fundamental research projects, they are attempting to develop an understanding of the biology of this pest ant as well as more immediate control solutions. Laboratory evaluations of commercial ant baits identified several products that quickly kill small colonies of the Caribbean crazy ant. Baits that contained insect growth-regulating active ingredients, pyriproxyfen or S-methoprene, resulted in average brood reductions of 80 percent. However, reductions were inconsistent, probably due to sporadic bait ingestion. New lures have been formulated with insect growth-regulating ingredients that exhibit more consistent consumption and are currently being evaluated. Insect growth-regulating baits can potentially be more effective against pest ants with large, interconnected colonies. A massive gene library sequencing approach was utilized to aid in the discovery of natural enemies and pathogens of these ants. No biological control agents for the Caribbean crazy ant are currently known, but the sequencing project identified several viruses with potential to serve as classical biological control agents or biopesticides.

Valles, S. M., Oi, D. H., Yu, F., Tan, X.-X., and Buss, E. A. 2012. Metatranscriptomics and pyrosequencing facilitate discovery of potential viral natural enemies of the invasive Caribbean crazy ant, *Nylanderia pubens*. PloS ONE 7(2): e31828. doi:10.1371/journal.pone.0031828.

Oi, D. H. 2012. Raves and rants about invasive crazy ants, pp. 11-12. In: Oliver, J. B. [ed.] Proceed. 2012 Imported Fire Ant Conf. April 16-18, 2012, Nashville, Tennessee. 109 pp.

PROBLEM STATEMENT 5C: *Potentially invasive ants (e.g. leaf-cutting ants and other fire ant species) could be introduced to the country, complicating already difficult ant control.*

The following locations have research projects addressing the issue identified under Problem Statement 5C:

- Imported Fire Ant and Household Insect Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.
- Biological Control of Pests Research Unit, National Biological Control Laboratory, Stoneville, Mississippi.

Problem Statement 5C: Select Accomplishments

Exotic species of ants in U.S. territory. Ants are easily transported invasive species. There is a better chance of stopping exotic, damaging species before rather than after they become more widely distributed. ARS scientists in Stoneville, Mississippi, conducted surveys of nurseries, railway terminals, ports, and recreational areas to develop new distributional records of several exotic ant species and two new State (Mississippi) records of introduced pest species. Two populations of the Caribbean crazy ant, *Nylanderia fulva*, were discovered in the fall of 2009 in Hancock County, Mississippi. Collaborators from Mississippi State University described the ant fauna of the Black Belt Prairie remnants in Mississippi and Alabama, helping to establish a baseline for this relatively undisturbed habitat. Unfortunately, six exotic ant species, including imported fire ants, were found to inhabit this ecologically sensitive habitat.

FIGURE 9: Yellow crazy ants cover this ground-nesting red-tailed tropic bird on Johnston Atoll.

ARS scientists from Gainesville, Florida, assisted with studies of the yellow crazy ant, *Anoplolepis gracilipes*, an invasive pest that infests Hawaii as well as many South Pacific islands and the Northern Territory in Australia. Yellow crazy ants have invaded Johnston Atoll National Wildlife Refuge, which is located about 825 miles southwest of Hawaii, and they are a threat to an essential breeding habitat for several species of seabirds. ARS provided guidance to the U.S. Fish and Wildlife Service on possible methods to suppress the ants. Recommendations for treatment protocols as well as evaluation of baits formulated specifically for the yellow crazy ant were developed. In addition, the yellow crazy ant was not detected in a survey during the site visit of three other islands that comprise the atoll. As a result of the collaboration between ARS and the Fish and Wildlife Service, there has been an observed reduction in ant populations and an increase in sea bird nesting at the refugee.

Oliver, J.B., Vander Meer, R.K., Ochieng, S.A., Youssef, N.N., Pantaleoni, E., Mrema, F.A., Vail, K.M., Parkman, P., Valles, S.M., Huan, W.G., and Powell, S. 2009. Statewide survey of imported fire ant (Hymenoptera: Formicidae) populations in Tennessee. Journal of Entomological Science 44(2):149-157.

Menzel, T.O., Cross, D.C., Nebeker, T.E., Caprio, M., and Chen, J. 2008. A survey of imported fire ant (Hymenoptera: Formicidae), species and social forms across four counties in east-central Mississippi. Midsouth Entomologist 1:3-10.

PROBLEM STATEMENT 5D**:** *Emerging household insect pests could become established as major problems in the United States.*

The following location has research projects addressing the issue identified under Problem Statement 5D:

- Imported Fire Ant and Household Insect Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.

Problem Statement 5D: Select Accomplishments

This problem was not addressed as originally intended. Although there were discussions about using RNAi technology against cockroaches and potentially other urban pests, ARS was not able to develop the anticipated cooperative arrangements with industry that would have aided this research.

APPENDIX 1

National Program 104 – Veterinary, Medical and Urban Entomology

ACCOMPLISHMENT REPORT 2007-2011

Research Projects in NP 104

Project Number	Project Title and Location
1275-32000-007-00D	DISCOVERY AND DEVELOPMENT OF CHEMICALS FOR INTEGRATED PEST MANAGEMENT OF BITING ARTHROPODS AND URBAN PESTS; Mark Feldlaufer (P), John Carroll, Joseph Dickens, and Kamal Chauhan; Beltsville, Maryland.
5430-32000-003-00D	INTEGRATED APPROACHES FOR PROTECTION OF ANIMALS FROM VECTOR-BORNE PATHOGENS; Lee Cohnstaedt (P), Barbara Drolet, William Wilson, D. Scott McVey, Dana Nayduch, Mark Ruder, and Robert Pfannenstiel; Manhattan, Kansas.
5440-32000-009-00D	DEVELOPMENT OF TOOLS FOR INTEGRATED PEST MANAGEMENT OF STABLE FLIES; David Taylor (P), Junwei Zhu, and Barbara Friesen; Lincoln, Nebraska.
6205-32000-031-00D	MINING THE GENOME OF *RHIPICEPHALUS MICROPLUS* TO DEVELOP NOVEL CONTROL TECHNOLOGY AND VACCINES; Felicito Guerrero (P), Kevin Tenmeyer, Robert Miller, Andrew Li, Adalberto Perez de Leon, Ronald Davey, Pia Untalan Olafson, John Goolsby, and Weste Osbrink; Kerrville, Texas.
6205-32000-033-00D	MOLECULAR BIOLOGY AND PHYSIOLOGY OF BITING FLIES AFFECTING LIVESTOCK; Andrew Li (P), Pia Untalan Olafson, Kevin Temeyer, Adelberto Perez de Leon, Felix Guerrero, and Kimberly Lohmeyer; Kerrville, Texas.
6205-32000-034-00D	BIOLOGY AND CONTROL OF TICKS OF VETERINARY AND HUMAN IMPORTANCE; Joe Pound (P), Ronald Davey, Kimberly Lohmeyer, Andrew Li, Robert Miller, Pia Untalan Olafson, Adalberto Perez de Leon, John Goolsby, Donald Thomas, and Weste Osbrink; Kerrville, Texas.
6205-32000-035-00D	HIGHER DIPTERA PESTS OF LIVESTOCK AND POULTRY: SCREWWORM FLIES; Steven Skoda (P), Muhammad Chaudhury, Agustin Sagel, and Pamela Phillips; Kerrville, Texas.
6402-22320-008-00D	BITING AND STINGING PESTS: ECOLOGY AND BIOLOGICALLY-BASED CONTROL; Jian Chen (P), Margaret Allen, Walker Jones, Xixuan Jin; Stoneville Mississippi.
6435-32000-012-00D	TERMITES: BIOLOGY, RISK ASSESSMENT AND SURVEILLANCE OF INVASIVE SPECIES; Alan Lax (P), Mary Cornelius, Matthew Tarver, Dunha Zhang, and Beverly Wiltz; New Orleans, Louisiana. (*TERMINATED IN 2012*)

Project Number	Project Title and Location
6435-32000-013-00D	TERMITES: CHEMICAL AND BIOLOGIAL CONTROL FOR INTEGRATED MANAGEMENT OF INVASIVE SPECIES, NEW ORLEANS, LOUISIANA; Alan Lax (P), Mary Cornelius, John Bland, Kumudini Meepagala, Weste Osbrink, and Maureen Wright; New Orleans, Louisiana. (*TERMINATED IN 2012*)
6435-32000-014-00D	COORDINATION OF NATIONAL TERMITE MANAGEMENT PROGRAM, NEW ORLEANS, LOUISIANA; Frank Guillot (P) and Alan Lax; New Orleans, Louisiana (*TERMINATED IN 2012*)
6615-32000-044-00D	BIOLOGY, GENOMICS, AND INTEGRATED PEST MANAGEMENT OF INVASIVE ANTS, Steven Valles (P), Robert Vander Meer, Sanford Porter, David Oi, and David DeWayne Shoemaker; Gainesville, Florida
6615-32000-045-00D	BITING ARTHROPODS: INTEGRATED PEST MANAGEMENT; Ulrich (Uli) Bernier (P), Gary Clark, James Becnel, Daniel Kline, Jerome Hogsette, and Kenneth Linthicum; Gainesville, Florida.
6615-32000-047-00D	HIGHER DIPTERA PESTS OF LIVESTOCK, POULTRY, AND HUMAN HEALTH: INTEGRATED PEST MANAGEMENT AND ADULT BIOLOGY; Jerome Hogsette (P), Gary Clark, Ulrich (Uli) Bernier, James Bechnel, Christopher Geden, and Daniel Kline; Gainesville, Florida.

APPENDIX 2

National Program 104 – Veterinary, Medical and Urban Entomology

ACCOMPLISHMENT REPORT 2007-2011

Publications by Research Projects

1275-32000-007-00D

DISCOVERY AND DEVELOPMENT OF CHEMICALS FOR INTEGRATED PEST MANAGEMENT OF BITING ARTHROPODS AND URBAN PESTS; Mark Feldlaufer (P), John Carroll, Joseph Dickens, and Kamal Chauhan; Beltsville, Maryland.

Bohbot, J.D. and Dickens, J.C. 2012. Odorant receptor modulation: Ternary paradigm for mode of action of insect repellents. Neuropharmacology 62.5: 2086-2095.

Carroll, J.F. and Kramer, M.H. 2012. Responses of Lone Star Tick (Acari: Ixodidae) nymphs to the repellent deet applied in acetone and ethanol solutions in in vitro bioassays. Journal of Entomological Science 47:193-196.

Bohbot, J.D., Fu, L., Le, T.N., Chauhan, K.R., Cantrell, C.L., and Dickens, J.C. 2011. Multiple activities of insect repellents on odorant receptors in mosquitoes. Medical and Veterinary Entomology. dx.doi.org/10.1111/j.1365-2915.2011.00949.x.

Feldlaufer, M.F. and Blomquist, G. 2011. Cuticular hydrocarbons from the bed bug C*imex lectularius* L.. Biochemical Systematics and Ecology 39:283-285.

Grant, A.J. and Dickens, J.C. 2011. Functional characterization of the octenol receptor neuron on the maxillary palps of the yellow fever mosquito, *Aedes aegypti.* PLoS ONE 6(6): e21785. doi:10.1371/journal.pone.0021785

Carroll, J.F., Benante, J.P., Kramer, M.H., Lohmeyer, K.H., and Lawrence, K. 2010. Formulations of Deet, Picaridin and IR3535 applied to skin repel nymphs of the Lone Star Tick (Acari: Ixodidae) for 12 hours. Journal of Medical Entomology 47(4):699-704.

Domingue, M.J., Kramer, M.H., and Feldlaufer, M.F. 2010. Sexual dimorphism of bed bug (*Cimex lectularis*) attraction and aggregation responses to cuticular extracts from nymph exuviae. Physiological Entomology 35(3):203-213.

Feldlaufer, M.F., Domingue, M.J., Chauhan, K.R., and Aldrich, J.R. 2010. 4-Oxo-Aldehydes from the dorsal abdominal glands of the bed bug (hemiptera: cimicidae). Journal of Medical Entomology 47(2):140-143.

Carroll, J.F., Hill, D.E., Allen, P.C., Young, K.W., Miramontes, E., Kramer, M., Pound, J.M., Miller, J.A., and George, J.E. 2009. The impact of 4-Poster deer self-treatment devices at three locations in Maryland. Vector-Borne and Zoonotic Diseases 9.4: 407-416.

Carroll, J.F., Pound, J.M., Miller, J.A., and Kramer, M. 2009. Sustained control of Gibson Island, Maryland, populations of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) by community-administered 4-Poster deer self-treatment bait stations. Vector-Borne and Zoonotic Diseases 9.4: 417-421.

Kramer, M., Weldon, P., and Carroll, J.F. 2009. Composite scores for concurrent behaviours constructed using canonical discriminant analysis. Journal of Animal Behavior 77(3):763-789.

Vogt, R.G., Miller, N.E., Litvack, R., Fandino, R.A., Sparks, J., Staples, J., Friedman, R., and Dickens, J.C. 2009. The insect SNMP gene family. Insect Biochemistry and Molecular Biology 39(7):448-456.

Carroll, J.F., Klun, J.A., and Kramer, M. 2008. Similarity in responses of laboratory-reared and field-collected lone star tick (Acari:Ixodidae) nymphs to repellents. Journal of Entomological Science 43(4):426-430.

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5430-32000-003-00D

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MINING THE GENOME OF *RHIPICEPHALUS MICROPLUS* TO DEVELOP NOVEL CONTROL TECHNOLOGY AND VACCINES; Felicito Guerrero (P), Kevin Tenmeyer, Robert Miller, Andrew Li, Adalberto Perez de Leon, Ronald Davey, Pia Untalan Olafson, John Goolsby, and Weste Osbrink; Kerrville, Texas

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MOLECULAR BIOLOGY AND PHYSIOLOGY OF BITING FLIES AFFECTING LIVESTOCK; Andrew Li (P), Pia Untalan Olafson, Kevin Temeyer, Adalberto Perez de Leon, Felix Guerrero, and Kimberly Lohmeyer; Kerrville, Texas

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BIOLOGY AND CONTROL OF TICKS OF VETERINARY AND HUMAN IMPORTANCE; Joe Pound (P), Ronald Davey, Kimberly Lohmeyer, Andrew Li, Robert Miller, Pia Untalan Olafson, Adalberto Perez de Leon, John Goolsby, Donald Thomas, and Weste Osbrink; Kerrville, Texas

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TERMITES: BIOLOGY, RISK ASSESSMENT AND SURVEILLANCE OF INVASIVE SPECIES; Alan Lax (P), Mary Cornelius, Matthew Tarver, Dunha Zhang, and Beverly Wiltz; New Orleans, Louisiana.

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BITING ARTHROPODS: INTEGRATED PEST MANAGEMENT; Ulrich (Uli) Bernier (P), Gary Clark, James Becnel, Daniel Kline, Jerome Hogsette, and Kenneth Linthicum; Gainesville, Florida.

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APPENDIX 3

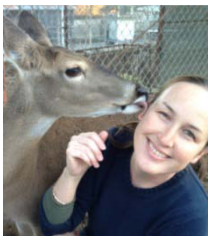
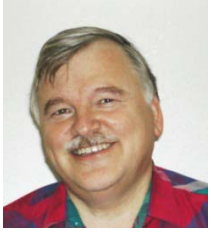
National Program 104 – Veterinary, Medical and Urban Entomology

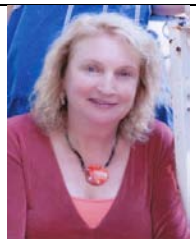
ACCOMPLISHMENT REPORT 2007-2011
Research Personnel by Location

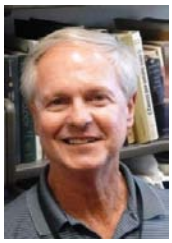
	John Carroll, Research Entomologist Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland
	Kamal Chauhan, Research Chemist Invasive Insect Biocontrol and Behavior Laboratory Beltsville, Maryland
	Joseph Dickens, Research Entomologist Invasive Insect Biocontrol and Behavior Laboratory Beltsville, Maryland
	Mark Feldlaufer, Research Entomologist Invasive Insect Biocontrol and Behavior Laboratory Beltsville, Maryland
	D. Scott McVey, Research Leader Arthropod-Borne Animal Diseases Research Unit Manhattan, Kansas

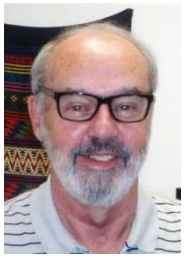
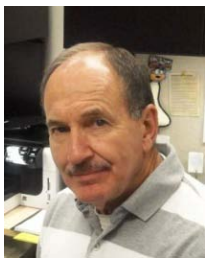
(photo not available)	**Lee Cohnstaedt, Research Entomologist** Arthropod-Borne Animal Diseases Research Unit Manhattan, Kansas
	Barbara Drolet Research Entomologist Arthropod-Borne Animal Diseases Research Unit Manhattan, Kansas
	William C Wilson, Research Microbiologist Arthropod-Borne Animal Diseases Research Unit Manhattan, Kansas
	David Taylor, Research Entomologist Agroecosystem Management Research Unit Lincoln, Nebraska
	Kristina Friesen, Research Entomologist Agroecosystem Management Research Unit Lincoln, Nebraska
	Junwei (Jerry) Zhu, Research Entomologist Agroecosystem Management Research Unit Lincoln, Nebraska

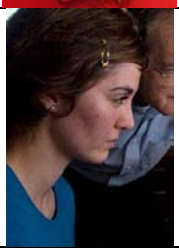
	Adalberto A Perez De Leon (Beto) **Laboratory Director** Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Steven Skoda, Research Leader Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Muhammad Chaudhury **Research Entomologist** Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Felix D. Guerrero, Research Physiologist (Insects) Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
(photo not available)	**Andrew Li, Research Entomologist** Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Kimberly Lohmeyer, Research Entomologist Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas

	**Pia Untalan Olafson** **Research Molecular Biologist** Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Weste Osbrink, Research Entomologist Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Joe (Mat) Pound, Research Entomologist Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	**Kevin Temeyer, Molecular Biologist** Knipling-Bushland U.S. Livestock Insects Research Laboratory Kerrville, Texas
	Ronald Davey, Research Entomologist Knipling-Bushland U.S. Livestock Insects Research Laboratory Mission, Texas
	John Goolsby, Research Entomologist Knipling-Bushland U.S. Livestock Insects Research Laboratory Mission, Texas
(photo not available)	**Donald Thomas, Research Entomologist** Knipling-Bushland U.S. Livestock Insects Research Laboratory Mission, Texas

	**Pamela Phillips, Physical Scientist** Screwworm Research Unit Pacora, Panama
	Agustin Sagel Screwworm Research Unit Pacora, Panama
	Walker Jones, Research Leader Biological Control of Pests Research Unit Stoneville, Mississippi
	Jian Chen, Research Entomologist Biological Control of Pests Research Unit Stoneville, Mississippi
	Stephen Duke, Research Leader Natural Products Utilization Research Unit Oxford, Mississippi
	Kumudini Meepagala, Research Chemist Natural Products Utilization Research Unit Oxford, Mississippi

	**Kenneth Linthicum, Center Director** Center for Medical, Agricultural and Veterinary Entomology Gainesville Florida
	Robert K. Vander Meer, Research Leader Imported Fire Ant and Household Insects Research Unit Gainesville, Florida
	David Oi, Research Entomologist Imported Fire Ant and Household Insects Research Unit Gainesville, Florida
	Sanford D. Porter, Research Entomologist Imported Fire Ant and Household Insects Research Unit Gainesville, Florida
	David DeWayne Shoemaker **Research Entomologist** Imported Fire Ant and Household Insects Research Unit Gainesville, Florida
	Steven M. Valles, Research Entomologist Imported Fire Ant and Household Insects Research Unit Gainesville, Florida

	**Gary Clark, Research Leader** Mosquito and Fly Research Unit Gainesville, Florida
	**Donald R. Barnard, Research Entomologist** Mosquito and Fly Research Unit Gainesville, Florida
	James Becnel, Research Entomologist Mosquito and Fly Research Unit Gainesville, Florida
	Ulrich (Uli) Bernier, Research Chemist Mosquito and Fly Research Unit Gainesville, Florida
	Christopher Geden, Research Entomologist Mosquito and Fly Research Unit Gainesville, Florida
	Jerome Hogsette, Research Entomologist Mosquito and Fly Research Unit Gainesville, Florida

	Daniel Kline, Research Entomologist Mosquito and Fly Research Unit Gainesville, Florida
	**Alexandra Chaskopoulou** **Research Entomologist** European Biological Control Laboratory Thessaloniki, Greece

APPENDIX 4

National Program 104 – Veterinary, Medical and Urban Entomology

ACCOMPLISHMENT REPORT 2007-2011

ARS Strategic Plan Alignment of NP 104 Research

RELATIONSHIP OF THIS NATIONAL PROGRAM TO THE ARS STRATEGIC PLAN

Outputs of NP 104 research support the actionable strategies associated with the performance measures shown below from the *ARS Strategic Plan for 2006-2011,* Strategic Goal 4: Enhance Protection and Safety of the Nation's Agriculture and Food Supply; Objective 4.2: *Reduce the number, severity and distribution of agricultural pest and disease outbreaks.* (www.ars.usda.gov/SP2UserFiles/Place/00000000/ARSStrategicPlan2006-2011.pdf)

Two performance measures set the targets for NP 104 research within the Strategic Plan:

Performance Measure 4.2.1: Provide scientific information to protect animals, humans, and property from the negative effects of pests, infectious diseases, and other disease-causing entities.

Target: Cumulatively, complete 10 research studies that have significant impact on the scientific community, leading to development of technologies for the integration of prevention and treatment strategies to manage top priority endemic and exotic threats to livestock, humans, and property.

Performance Measure 4.2.2: 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.

Target: Cumulatively, transfer five technologies to the commercial and government sectors.

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