Veterinary, Medical, and Urban Entomology (NP 104)

Annual Report for 2009

2, 4, 6, 8: Protecting two- and four-legged animals from the six- and eight-legged arthropods

Background:

National Program (NP) 104 involves efforts in 10 units at 8 different locations performed by more than 80 scientists. The subjects of investigation concern improvements in integrated pest management for termites, ants, mosquitoes, biting midges, sand flies, biting and filth flies, screwworm flies, bed bugs, and ticks. We aim for 30 percent of our work to be basic, in the sense that the results may not have direct application but have the potential to truly innovate the field. The other 70 percent of the work focuses on solution of particular problems, including developmental work beyond the research phase.

This year, NP 104 completed the review process by the Office of Scientific Quality and Review. Certification of project plans that had been prepared during a 2-year process initiates a new 5-year cycle. Thanks to the careful efforts of our lead scientists and Area Offices, the scores of the projects were better than the peer review. Some of the changes in program direction include creation of a new project dedicated to control of muscoid Diptera (higher flies), addition of bed bugs as a target, and consolidation of efforts at the Beltsville laboratory. We have seen much greater collaboration between laboratories working on mosquitoes (Beltsville, Gainesville, Laramie, and Stoneville) and on flies (Gainesville, Lincoln, Kerrville, and Pacora).

We also experienced a new partnership with the University of Florida and the USDA ARS European Biological Control Laboratory. Their work in Thessaloniki, Greece, contributed to development of new aerial spray techniques for adult mosquitoes. We are hoping that this collaboration will result in more work in the years to come as an extension of the mission of this facility into medical and veterinary entomology.
The South American Biological Control Laboratory, Hurlingham, Buenos Aires, Argentina, has long collaborated with the Imported Fire Ant and Household Insects Research Unit in Gainesville, Florida, on collection and evaluation of parasites and parasitoids of fire ants.

Other international work included development of projects on Rift Valley fever and sand fly control in Kenya and Egypt, fly control in Egypt, tick control in Mexico, anti-tick vaccine development in Australia and Brazil, and our continuing work in Panama on screwworm fly. Scientists consulted on fire ants in China, New Zealand, and Australia and with the World Health Organization.

NP 104 was also a contributor to a report on research gaps on the effects of climate change on public health, with an emphasis on vector-borne diseases. The results of this report made it clear that the contributions of NP 104 will be important in protecting

Americans from an increasing threat due to greater frequency of severe weather events and generally warmer conditions. Risk modeling on Rift Valley fever, West Nile fever, and screwworm fly has already contributed solutions to some of these problems.

With the certification of the new project plans, NP 104 is initiating its Action Plan for 2009–2013. This plan describes the program's rationales and goals in detail. It is available at (http://www.ars.usda.gov/SP2UserFiles/Program/104/NP104ACTIONPLANFY09-FY13Final.pdf).

NP 104 Events in 2009:

We welcomed the following new scientists to NP 104:

Mat Tarver, Ph.D. as a scientist at the Formosan Subterranean Termite Research Unit, New Orleans, Louisiana.

Augustín Sagel as a new scientist at the Screwworm Research Unit, Pacora, Panama.

Liming Zhao, Ph.D., as a post-doc at the Biological Control of Pests Research Unit, Stoneville, Mississippi.

Alan J. Grant, Ph.D., as a post-doc at the Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Shyam Shirali, Ph.D., as a post-doc at the Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Monique Coy, Ph.D., as a post-doc at the Mosquito and Fly Research Unit, Gainesville, Florida.

Clare Allen, Ph.D., as a post-doc at the Imported Fire Ant and Household Insect Research Unit, Gainesville, Florida.

The following NP 104 scientists received external grants for their research:

Uli Bernier, Mosquito and Fly Research Unit, Gainesville, Florida, received $139,000 from the Natick Soldier Center to test insecticide-treated uniforms.

DeWayne Shoemaker and Steven Valles, Imported Fire Ant and Household Insects Research Unit, Gainesville, Florida, were awarded an INFECTIGEN grant of $100,000 for 2 years in partnership with Lausanne University, Switzerland. The subject of the study is metagenomics of fire ants.

DeWayne Shoemaker, Imported Fire Ant and Household Insects Research Unit, Gainesville, Florida, was awarded a CSREES NRI grant of $719,196 for 4 years in partnership with Pennsylvania State University and Lausanne University, Switzerland. The subject of the study is development of new functional genomic resources for the fire ant *Solenopsis invictae.*

Sanford Porter, Imported Fire Ant and Household Insects Research Unit, Gainesville, Florida, received $24,000 from APHIS to facilitate collection, rearing, and evaluation of new species of fire ant-decapitating flies from South America for field release and transfer to the APHIS mass rearing program.

Jeanne Howell and Pia Olafson, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas, were awarded a NIFA AFRI grant of $450,000 for 3 years in partnership with Northern Arizona University. The subject of the project is population genetics of the cattle fever tick and its infection with *Babesia*.

Adalberto Pérez de León, Kim Lohmeyer, Jeanne Howell, Mat Pound, and Ron Davey, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas, were awarded a NIFA AFRI Rapid Response Food and Agricultural Science for Emergency Issues Program grant of $250,000 for 2 years in partnership with Texas A&M University. The subject of the project is demonstration of integrated eradication of cattle fever tick on deer and cattle.

Adalberto Pérez de León, Pia Olafson, and Felix Guerrero, Knipling-Bushland U.S. Livestock Research Laboratory, Kerrville, Texas, are working with Renato Andreotti of Embrapa (the Brazilian equivalent of ARS) on development of anti-tick vaccines. Dr. Andreotti's project, "Abordagem molecular no dagnóstico da resistência e no controle do carrapato-do-boi," was funded at the equivalent of $68,000 for 2 years. The project includes a 3-month visit by Dr. Andreotti to Kerrville.

Kristine Bennett, Arthropod-Borne Animal Diseases Research Laboratory, Laramie, Wyoming, and Kenneth Linthicium, Center for Medical Agricultural and Veterinary Entomology, Gainesville, Florida, were awarded $400,000 for up to 5 years by the Department of State Biodefense Engagement Program in partnership with the Kenya Medical Research Institute and the International Livestock Research Institute. The subject of the project is ecology and control of Rift Valley fever.

Joseph Dickens, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland, received $250,000 from the Department of Defense Deployed Warfighter Protection Program for the study of mosquito olfaction.

Kamlesh Chauhan, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland, received $400,000 from the Department of Defense Deployed Warfighter Protection Program for the development of new insecticides and repellents.

Gary Clark and staff, Mosquito and Fly Research Unit, Gainesville, Florida, received $1,500,000 from the Department of Defense Deployed Warfighter Protection Program for the development of better methods for protection of deployed military personnel from insect-borne diseases.

Kumidini Meepagala and others at the Natural Products Utilization Research Unit, Oxford, Mississippi, received $280,000 from the Department of Defense Deployed Warfighter Protection Program for the development of natural products as insecticides and repellents.

Andrew Li, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas, received $30,000 from the Department of Defense Deployed Warfighter Protection Program for the development of better products to control sand flies.

Felix Guerrero, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas, received $200,000 from APHIS International Services for development of a male-only strain of the screwworm fly.

We congratulate:

Andrew Li, Ph.D., Knipling-Bushland U.S. Livestock Insect Research Laboratory, for receiving an Administrator's ARS Research Associate Program award for the project, Novel Stable Fly Control Through Interruption of Mating and Egg-Laying Behaviors.

Jian Chen, Ph.D., Biological Control of Pests Research Unit, Stoneville, Mississippi, for receiving an Administrator's ARS Research Associate Program award for the project, Functional Genomics Investigation of the Biosynthesis of Hydrocarbons in Ants.

Uli Bernier, Ph.D., for receiving a Technology Transfer Award from USDA ARS for development of a protocol to test the efficacy of permethrin-treated uniforms to prevent mosquito bites.

Gary Clark, Don Barnard, and Uli Bernier of the Mosquito and Fly Research Unit, Gainesville, Florida, for preparation of guidance documents for testing repellents and insecticides according to World Health Organization standards.

Jerome Klun, Ph.D., on his retirement from the Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland.

Will Reeves, Ph.D., on accepting a position with the U.S. Army Center for Health Promotion and Preventive Medicine.

Douglas Streett, Ph.D., on accepting a position with the U.S. Forest Service.

J.T. Vogt, Ph.D., on accepting a position with Black Pest Prevention, Inc.

We honor the following NP 104 scientists who were deployed on active duty from their military reserve positions:

CPT Seth Britch, Ph.D., U.S. Army Medical Service Corps, from the Mosquito and Fly Research Unit, Gainesville, Florida.

LCDR Robert Miller, Ph.D., U.S. Navy Medical Service Corps, from the Knipling-Bushland U.S. Livestock Insect Research Laboratory, Cattle Fever Tick Laboratory, Mission, Texas.

Technology Transfer:

One of the ways in which ARS scientists provide products that solve agricultural problems is through direct interactions with the public and industry. Five websites within NP 104 were managed, providing information directly to those who need to control fire ants and termites. Inventions from the program resulted in 2 new patent applications, 1 invention disclosure, and 5 new research agreements with industry.

The inventions most ready for development are advertised to industry as potentially valuable licenses (http://www.ars.usda.gov/business/availtechs.htm). NP 104 currently lists the following:

Natural Mosquito and Tick Repellent
Promising Biopesticide for Fire Ant Control
Improved Technique for Protecting and Treating Deer Against Ticks
New Method for Controlling Subterranean Termites
New Method for Developing Molecular Pesticides
Environmentally Safe Mosquito Attractant
Technologies to Control Subterranean Termites
New Water-Resistant Ant Bait
Method to Prepare a Natural Mosquito and Tick Repellent from Pine Oil
New Organic Pest Control for Flies and Ticks
Arthropod-Repelling Compounds
Portable Deer Lift
Technology to Help Develop Vaccines for Protecting Cattle Against Scabies
Repellents for Ants
Biological Control for Mosquitoes and Other Insects
Device and Method for Application of Collars to Animals

The publication of the comprehensive report on area-wide testing of the “4-Poster” device for control of deer ticks and Lyme disease was another form of technology transfer. This report appeared as a special volume of *Vector Borne and Zoonotic Diseases* (Volume 9, No. 4) and documented how the use of this ARS-invented device can reduce tick populations and prevalence of the pathogen that causes Lyme disease. The 7-year project involved many collaborators led by ARS.

Notable Accomplishments:*

First stereospecific mosquito odorant receptor characterized. There is a need to develop newer, more effective methods to protect humans from blood-sucking insects such as mosquitoes. Biological work at a basic level produces results that may lead to truly innovative solutions to this problem. In this accomplishment, the Invasive Insects Biocontrol and Behavior Laboratory, Beltsville, Maryland, developed methods for cloning odorant receptor proteins from mosquitoes and expression of the odorant receptors in the membranes of frog eggs. Carefully placed electrodes drew a very small voltage across the membrane, and the resulting current of electricity was monitored. The voltage potential was disrupted when odor chemicals washed across the proteins in the membrane, depolarizing it. In this way, it was possible to characterize the test proteins in the frog egg. One odorant receptor responded specifically to 1 of 2 mirror image forms of a compound; this mirror image is an important attractant for blood-feeding insects. This was the first mirror image-specific odorant receptor discovered and characterized for an invertebrate. This discovery will lead to improvement of the molecular design of chemicals that alter the behavior of insects, creating products for more efficient control of mosquitoes and other biting pests.

Mosquito virus infection influences effectiveness of insect repellents. Insect repellents are used to prevent bites and subsequent infection with mosquito-borne pathogens, but their effectiveness on the infected mosquitoes that transmit the pathogens was unknown. Funded in part by the Department of Defense Deployed Warfighter Protection Program, scientists at the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, collaborated with scientists at the U.S. Army Medical Research Institute for Infectious Diseases to study this problem. Venezuelan equine encephalitis virus (VEEV)-infected *Aedes taeniorhynchus* mosquitoes and their “sham-infected” siblings—inoculated with VEEV or diluent, respectively—were compared. It was found that infected mosquitoes bit a repellent-treated (DEET) hamster long 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. The results of this work show that repellents will have to be redesigned to be sure that they are effective in deterring bites from the very infected biting insects, which are the most important to deter.

Effects of bluetongue virus infection on feeding by the biting midge vector. *Culicoides sonorensis* (Diptera: Ceratopogonidae) is the primary vector of bluetongue virus (BTV), which affects the health of livestock and movement of animals in North America and Europe. Scientists at the Arthropod Borne Animal Diseases Research Laboratory, Laramie, Wyoming, performed experiments to determine whether infection with BTV affects blood-feeding behavior. Midges were injected with either BTV-infected or BTV-free cell lysates and were then allowed to blood feed on sheep for short (10-minute) or long (60-minute) periods of time. Midges that had been infected for 7 days did not feed as rapidly as did uninfected midges or as rapidly as midges that had been infected only 2

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* Components are from the 2005–2009 Action Plan: 1. Ecology and Epidemiology; 2. Detection and Surveillance Technology; 3. Biology and Physiology; 4. Control and Technology.

or 4 days. One direct consequence of this research involves risk assessment. Estimates of transmission risk in a particular location would be affected if infected midges required a longer time to feed, affecting economic threshold calculations for these pests.

Salivary gland hypertrophy virus of house flies. House fly control with chemicals and sanitation is often inadequate for a number of reasons that are beyond the control of the farmer. Researchers at the Mosquito and Fly Research Unit, Gainesville, Florida, are working on innovative methods for fly control. They are investigating natural enemies of flies, including salivary gland hypertrophy virus (SGHV). This virus belongs to a novel group of viruses that infect salivary glands and block egg production in flies. The scientists performed a field survey that showed the wide distribution of the virus on Danish dairy farms, with maximum infection rates of about 10 percent of the flies. Transmission studies indicated that the virus was specific to house flies, an important finding because some related species are beneficial in some situations. Danish virus isolates had somewhat higher virulence in laboratory 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.

Origin of the invasive Formosan subterranean termite. The Formosan subterranean termite (FST) was introduced into the U.S. from Asia in the 1940s. Since then, it has developed into a devastating pest of wooden structures and living trees in the southeastern U.S. The Formosan Subterranean Termite Research Unit, New Orleans, Louisiana, used cutting-edge genetic techniques to determine the precise source of the invasive populations. Twenty-eight FST colonies were collected between 2004 and 2008, including 21 colonies from Mississippi, 6 from Louisiana, and 1 from China. Two genotypes of FST were identified in Mississippi. The first one, GA type, was identical to those reported previously in Georgia, Louisiana, Alabama, and other infested States. The second one, AT type, was identified for the first time in the southeastern U.S. Sequence identity of the AT type of FST with those reported mainly in China provided evidence of at least two introductions of FST to the U.S. The termites in Mississippi are potentially transported from the port cities in Mississippi along the Gulf of Mexico; numerous infestations have been observed along transportation corridors via Interstate 59 in Mississippi and Highway 11, as well as in a parallel railway from New Orleans, Louisiana, to Meridian (Lauderdale County), Mississippi. Despite the abundance and widespread distribution of FST in Mississippi, the genetic diversity is very limited. Twenty colonies collected in that State had the identical GA genotype, and there is only a recent introduction of the AT genotype. The fact that there were only two introductions of FST raises confidence in the use of control techniques that target specific gene sequences. This knowledge also enables detection of additional introductions and when they happened.

Residual barrier treatments for control of mosquitoes and sand flies. It is not always possible to target the larvae of biting flies for control. For example, phlebotomine sand flies that transmit leishmaniasis to American military personnel develop in a wide variety of sites in the soil that are not easy to detect or treat. Mosquitoes may develop from larvae located in habitats at a distance from the area where people are being bitten, and those larval habitats may not be accessible. Scientists at the Mosquito and Fly Research Unit, Gainesville, Florida, have been working to optimize techniques for application of insecticides to vegetation and other materials in order to create a barrier to adult biting fly movement. Four styles of U.S. military desert and woodland camouflage netting and tent systems, any of which could be routinely provided to current U.S. military units, were treated with one of two synthetic pyrethroids (bifenthrin and lambda-cyhalothrin) and set out in two desert environments. In addition, naturally occurring desert xeric vegetation in the Coachella Valley site in California was treated with a residual barrier of bifenthrin on two occasions. One cold-mist conventional backpack sprayer was used for all treatments of artificial substrates, but a variety of cold-mist and electrostatic sprayers was used for the two vegetation treatments. Efficacy of barriers was assessed in the field with a schedule of regular overnight mosquito population sampling within and outside of treated and untreated netting perimeters using a variety of traps. Efficacy of treated barriers was assessed in the laboratory by measuring mortality in bioassays on samples of vegetation or material using colony-reared female mosquitoes. Overall results from vegetation treatments indicated a significant reduction in mosquitoes in field counts and laboratory assays for up to 1 month; overall results of material treatments indicated significant reduction of mosquitoes or sand flies in field counts and laboratory assays for up to 8 months. Barrier treatments in a desert environment should provide considerable protection from mosquitoes and sand fly vectors of leishmaniasis.

Bed bug chemicals identified. Bed bugs have become a common problem in the U.S., increasing in frequency during the last 10 years. The causes for this resurgence are probably multiple, including more movement of people from heavily infested areas of the world, increases in the proportion of people living in multi-family housing, and widespread resistance to pyrethroid insecticides. The Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, Maryland, is working on better ways to detect and control bed bugs as part of urban integrated pest management. Several new control methods designed to attract and trap bed bugs incorporate chemicals produced by the bugs. ARS identified 2 new compounds from the glands of bed bugs that may be important in their chemical ecology. Incorporation of these compounds into existing traps may increase the traps' efficiency, thereby achieving better bed bug control with less use of pesticides.

Natural products as termiticides. Economically, termites are the most important insect pest in the U.S., but relatively few active ingredients are registered for their control. The Formosan Subterranean Termite Research Unit, New Orleans, Louisiana, worked with the Natural Products Research Unit, Oxford, Mississippi, to find new, safe, active ingredients for termite control. Researchers discovered that plant-derived chromenes show termiticidal properties. Analogs of this natural product are even more active against the Formosan subterranean termite and show activity against other insect species as well. A patent for these compounds has been filed. Chemical modification of the parent compound has improved termiticidal activity. Chemicals resulting from this research may be useful for controlling termites or providing clues for new chemistries that could be developed as termiticides that are safe for the environment and for applicators.

Control of the cattle fever tick. Eradication of the cattle fever tick (vector of bovine babesiosis) from the entire country was accomplished between 1907 and 1943 by systematic treatment of cattle across the entire southern U.S. Since 1943, the tick has been kept out of the U.S. by application of a rigorous quarantine program that inspects cattle imported from Mexico, rounds up stray cattle that wander across the Rio Grande River, and imposes treatment regimens on producers who have infested cattle on their property. A narrow quarantine zone in 5 counties of southernmost Texas was for many years adequate for active surveillance. During the last 5 years, the number of infestations occurring outside the quarantine zone has increased alarmingly, reaching levels not seen since 1973. Unlike the situation in Mexico, the ticks within the U.S. are usually susceptible to acaricides, suggesting that the problem is ecological rather than technical. It is strongly suspected that huge increases in the population of white-tailed deer, and possibly in exotic ungulate species, has been the cause of the expanding populations of cattle fever ticks in Texas. Scientists at the Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas, have been working to develop methods to treat ticks on wild deer. During 2009, field trials of technologies under development to prevent feral swine and javelina from accessing and destroying ARS-patented "4-Poster" Deer Treatment Bait Stations were completed, with varying degrees of positive results. Initial attempts to see whether access to a "4-Poster" could be prevented by raising them off the ground on metal posts proved insufficient to prevent access. 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, which 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 2 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 the electric fence wire and then rapidly retreated. The swine exclusion device was also used to protect a new deer-collaring device and the recently commercialized "2-Poster" Deer Treatment Feeder Adapters. Development and refinement of these tools will enable producers to control cattle fever ticks on wild ungulates as well as on their cattle.

Ant ecology related to fire ant infestations. Imported fire ant species have replaced native ant fauna in much of the southern U.S., harming natural ecology and making it more difficult to control fire ants. Scientists at the Biological Control of Pests Research Unit, Stoneville, Mississippi, have been studying the basic ecology of ants in order to assess how native ants influence the distribution of invasive ants. Preliminary surveys of ants at select nurseries in Mississippi that obtain 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 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 initiated to measure changes in the ant community in prairie remnants where eastern red cedar is being removed by different methods. The invasive Asian needle ant, *Pachycondyla chinensis*, was discovered nesting near Birmingham, Alabama, in a natural area. Surveys for ants in three Georgia Sandhill Natural Areas were also completed. A survey of ants in the Great Smoky Mountains National Park was initiated as part of the All Taxa Biodiversity Inventory. The range of the hybrid (black x red) imported fire ant (HIFA) is continuing to expand. Because polygyny (a colony with multiple queens) and higher population density are typical of HIFA, the fire ant problem will probably get worse as the black imported fire ant is replaced by the hybrid. In addition, the HIFA queens received multiple inseminations, possibly leading to more rapid adaptation than observed for either the black or the red fire ants. Understanding the movement of ant species is the foundation for managing them to achieve a situation closer to the natural balance prior to the importation of fire ants.