NATIONAL PROGRAM 104

Veterinary, Medical, and Urban Entomology





ACCOMPLISHMENT REPORT: 2002 – 2006

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Introduction

This Accomplishment Report summarizes the scientific and product-development achievements of the USDA Agricultural Research Service's National Program 104, Veterinary, Medical, and Urban Entomology for the period January 2002 through December 2006. National Program 104 emphasizes protection rather than production, in that the ultimate aim of all of the research is improvement of integrated pest management (IPM). The goals of the program have evolved over the years in an attempt to match actual national problems and current scientific advances. The last set of goals was written in the Action Plan (http://www.ars.usda.gov/research/programs/programs.htm?np_code=104&docid=274) published in August 2003. That Action Plan was the result of input from two workshops attended by stakeholders who had an interest in the direction of ARS' research, organizations like the National Cattlemen's Beef Association, the Centers for Disease Control and Prevention, and the National Pest Management Association. Following a structured format, the stakeholders provided their viewpoints on the problems that most affected their activities and the ways in which ARS research might help them. The NP 104 Action Plan is used as a guide for the National Program Leader to write a series of "Program Direction and Resource Allocation Memoranda" (PDRAMs), an important component of which is a series of objectives that guides the research accomplished in the laboratory. The individual laboratories respond to the PDRAMs by writing Project Plans, which are peer-reviewed by independent panels, revised, and then used as the guides to research within each research project for the next five years. The progress achieved in attaining the Action Plan goals is assessed by an external retrospective assessment panel.

This report summarizes selected accomplishments that resulted from the ensuing research. It is organized by the Program's major components as outlined in the Action Plan:

Component 1: Ecology and Epidemiology
Component 2: Detection and Surveillance Technology
Component 3: Biology and Physiology
Component 4: Control Technology

A few words are necessary about the conceptual framework and scope of the program. Integrated Pest Management is the main subject of all of the research in this program. Taken in a broad sense, IPM also describes those practices that target pathogen transmission, as well as practices that target the arthropod pest itself. There are many ways to describe IPM, but for the purposes of this report, the elements of IPM are considered to be risk assessment, surveillance, control, and monitoring. Risk assessment answers the questions of whether or not there is a problem and where that problem occurs, usually from existing sources of information. Surveillance is the process of on-the-ground measurements to provide targeting information for control efforts. Arthropod control includes a wide variety of techniques which, at their best, are applied in an integrated fashion to achieve the most efficiency with the least possibility of environmental or occupational hazard. Finally, monitoring is the continuous process of

assessing the success of control measures in order to validate sustained effort and to detect any changes that might require additional action. These four elements of IPM will be used throughout this report in order to emphasize the relationship between the research and practical, operational entomology.

Accomplishments included in this report represent the majority of individual research projects that resulted in peer-reviewed publications. Recent work, especially that accomplished in 2006, is not necessarily connected to a publication. A few publications from 2007 are included, since they represent work completed in 2006. *Appendix 1* is a comprehensive bibliography of peer-reviewed publications, including book chapters and reviews. Many of the references are cited in the text of the report, but others are not. The full text of all of the articles is available on an ARS SharePoint site found at the following URL (https://arsnet.usda.gov/sites/NPS/APP/NP104%20Publications/).

ARS encourages its scientists to have impact not only through addition to the body of scientific knowledge, but also through product development and application. *Appendix 2* summarizes some of those accomplishments, though many details must be omitted due to proprietary interests. The ARS Office of Technology Transfer (http://www.ars.usda.gov/Business/Business.htm) can assist those interested in arranging partnerships with our researchers.

Background

Agriculture depends on people, animals, and plants. National Program 104 solves problems that affect people and animals, protecting them from the insects and ticks that cause direct injury or disease. ARS has a long history of achievement in this field. In fact, the first discovery of a disease-causing pathogen transmitted by an arthropod was performed by the microbiologist, Theobald Smith, in 1889-1893 while working for the U.S. Department of Agriculture. He found that Texas cattle fever was caused by a protozoan parasite in their blood, which was transmitted between cattle by a particular kind of tick. Smith's discovery was well-documented and often discussed at the time, leading to the astoundingly significant association of mosquitoes with transmission of malaria, yellow fever, and many other arboviruses such as those causing dengue and West Nile Virus. Smith's discovery also led to an eradication campaign started in the early 1900s that continues to this day, almost eliminating the threat of Texas cattle fever in our country. The importance of mosquitoes as vectors of important diseases caused a renewed interest in accurate taxonomy and, again, USDA was at the center of activity. L.O. Howard, H.G. Dyar, and F. Knab produced the definitive scholarly work on mosquitoes of the Americas, with Knab completing the manuscript in 1918 as he was dying from sand fly-borne leishmaniasis. In 1942 the War Department called on the USDA to develop new insecticides and repellents to protect military personnel from the ravages of scrub typhus and malaria, particularly in the Pacific Theater. Scientists at the Orlando laboratory eventually screened over 20,000 compounds and produced a series of compounds that could be applied to the skin or clothing. Before the introduction of chloramphenicol in 1949, scrub typhus was almost a death sentence and the repellents dimethyl phthalate, ethyl hexanediol, and benzyl benzoate were all that stood between soldiers and this infection. Thanks to USDA scientists Carroll Smith and Harry Gouck,

the screening effort in the 1950s found that Deet was a highly effective insect repellent. Deet remains the standard repellent active ingredient today. ARS also developed the current repellent treatment for military uniforms during the 1980s. The Department of Defense recently again asked ARS to produce better products to protect the U.S. military, as part of the DoD funded research program on "Deployed War-Fighter Protection (DWFP) from biting insects and associated diseases, so the agency has responded with a major effort under Component 4 below.

ARS research on the invasive fire ant in Gainesville, FL led to continuous breakthroughs in control methodology and control agents beginning in the late 1940s. These results led to a 10-fold decrease in the amount of heptachlor needed to control fire ants and, more importantly, the development of toxic bait methodology that takes advantage of the effective foraging ability of fire ants. The first toxic bait was developed in the early 1960s, using Mirex® as the active ingredient. This material controlled fire ants at a rate of only 1.5g active ingredient per acre. Persistent residues of Mirex® led EPA to cancel all registrations effective in 1978, leaving the public with no EPA registered fire ant toxic baits. ARS responded by screening thousands of compounds for the special delayed-acting requirements of fire ant bait toxicants resulting in the discovery, development and EPA registration of Hydramethylnon® in 1980. This was accomplished only two years after the registration of Mirex® was cancelled. Subsequently, other bait toxicants were discovered and registered providing the public with a variety of choices for fire ant control.

No discussion of ARS achievements would be complete without mentioning the research that led to the defeat of the screwworm fly. This fly actively seeks even the smallest wounds in cattle, lays its eggs, and then causes what is often mortal injury to the animals. In 1950, E.F. Knipling began an effort to release sterile males of the fly over huge areas, preventing the females from laying fertile eggs. This technique has been refined greatly over the years, but it continues and has successfully eradicated the fly as far south as Panama. The benefits from this program are almost incalculable, considering the economic benefit to the American beef industry, the relief from animal suffering, and the benefit to small landholders in Mexico and Central America.

Laboratories and Acronyms

During the 2002 – 2006 reporting period, National Program 104 had programs at the following laboratories:

ABADRL:	Arthropod-Borne Animal Diseases Laboratory, Laramie, Wyoming
APDL:	Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Beltsville Area Research Center, Beltsville, Maryland
APMRU:	Areawide Pest Management Research Unit, Southern Plains Area Research Center, College Station, Texas
BCPRU:	Biological Control of Pests Research Unit, Jamie Whitten Delta States Research Center, Stoneville, Mississippi
CAIBL:	Chemicals Affecting Insect Behavior Laboratory, Plant Science Institute, Beltsville Area Research Center, Beltsville, Maryland

FSTRU:	Formosan Subterranean Termite Research Unit, Southern Regional Research Center, New Orleans, Louisiana (satellite location at Poplarville, Mississippi)
IFAHIRU:	Imported Fire Ant and Household Insects Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
KBUSLIRL:	Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas (satellite location at Moore Field, Texas)
MFRU:	Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
MLIRU:	Midwest Livestock Insects Research Unit (now the Agroecosystem Management Research Unit), The Lincoln Location, Lincoln, Nebraska
SRU:	Screwworm Research Unit, Pacora, Panama (satellite location at Tuxtla-Gutierrez, Mexico)

Component 1: Ecology and Epidemiology

A thorough understanding of the habits of harmful arthropods is basic to the success of the other three components of NP 104. These are predominately observational and experimental studies done in the field by biologists trying to understand whole populations and their interactions with livestock or humans. For example, it is essential to know not only which mosquitoes are capable of transmitting West Nile virus, but also which species are responsible for transmission to horses and humans, where they breed and when they bite. It is only with this information that surveillance and control will be effective. How invasive species compete for local ecological niches also influences control strategies. For example, the diversity of native ant species is diminished when fire ants invade an area. Control efforts aimed at fire ants should ideally leave the environment suitable for recolonization of native ants. The underground colonizing behavior of the Formosan subterranean termite is poorly understood, making the placement of toxic baits inefficient.

From the standpoint of IPM, the research results in Ecology and Epidemiology most commonly facilitate the function of risk assessment. It is through an understanding of the distribution and interactions of vectors that the most accurate estimates of risk can be achieved. On the one side of the equation are the pests and all the variety of factors that affect their impact on humans and animals: species identification, longevity, host preference, capacity to damage infrastructure, infection rate, genetic diversity, mobility, etc. On the other side of the equation are the myriad potential influences on those pests, including geographic location, landscape, climate, weather history, human intervention, etc. Sometimes those influences on the pest are easier to measure than the pests themselves (e.g., remote sensing of vegetation data). In other situations, the challenge to accurate risk assessment is measurement of the most relevant aspect of the pest population (e.g., longevity compared to raw abundance). Accurate risk assessment can inform operational entomology regarding the existence of a problem in any given area and it's severity. Subsequent entomological intervention is more likely to be effective when the right equipment, materials, and labor are concentrated in the right area. From a research standpoint, an understanding of the risk leads to development of the most appropriate surveillance, control, and monitoring.

Component 1 Goals:

1.1 Identify Aspects of Arthropod Behavior Vulnerable to Control.

Summary of Accomplishments:

- The distribution of ticks within the woodland habitat identified priority areas for treatment.
- Geographic distribution of tick species are changing, resulting in new threats to health.
- Birds can exercise effective defensive behavior against mosquitoes, altering estimates of mosquito longevity and consequent risk assessment.
- Most *Culex pipiens* feed in the canopy of the urban forest, at least in the northeastern United States, implying that surveillance and adult control should address this habitat.

Impact:

This research makes it possible to improve the accuracy of sampling the vectors of Lyme disease and of West Nile virus. More accurate risk assessment and surveillance will have the double benefit of reducing treatment costs and improving treatment efficiency.

Ticks

Focal and geographic distributions determine where humans and livestock are going to encounter vector species. Host-seeking *Ixodes scapularis* nymphs were occasionally found on tree trunks and those found were <1 m above ground level, consistent with the association of this life stage with leaf litter and low growing vegetation. Interestingly, half of the *I. scapularis* nymphs found on leaf litter within 1 m of tree trunks were on the north side of the trunks, where they were presumably more protected from summer heat and desiccation (Carroll 2002c). Although *Ixodes scapularis* is usually associated with large populations of deer, a situation was observed at the National Institute for Science and Technology, Montgomery County, Maryland, where the ticks were scarce in spite of very large numbers of deer. The severe habitat degradation caused by the over abundant deer may have reduced populations of rodents and birds that serve as hosts for larval and nymphal ticks, thereby minimizing tick numbers (Carroll and Cyr, 2005). *Amblyomma americanum* has expanded its range north along the coastal plain of the Mid-Atlantic states, demonstrating the dynamic nature of tick distributions (Carroll, 2007).

Mosquitoes

Studies of the defensive behavior of chickens against mosquitoes showed that night-feeding mosquitoes (*Culex nigripalpus*) were eaten by chickens less often than day-feeding mosquitoes (*Aedes albopictus*), conferring a significant survival advantage to the night feeding species. Low mosquito abundance may give a survival advantage to individual females seeking a blood meal, since chicken defensive behavior was much less efficient when there were fewer mosquitoes. These observations help explain some epidemiological patterns of zoonotic pathogens, suggesting that defensive behavior may shorten longevity and actually damp down transmission cycles when mosquito

populations increase (Kobylinski, et al., 2007). In Connecticut, most West Nile virus was acquired by *Culex pipiens* in the canopy of trees, presumably where birds roost and mosquitoes feed at night. This study showed that over 85% of *Culex pipiens* were collected from the canopy compared to the ground (Anderson, et al., 2004).

1.1.1 Characterize the oviposition behavior of mosquitoes and midges and isolate environmental factors that attract or repel.

Summary of Accomplishments:

- Oviposition attractant chemicals may contribute toward more accurate surveillance and more targeted control of West Nile virus vectors.
- Oviposition repellents may have a role to play in mosquito IPM.
- The ecology of bluetongue virus transmission depends on the distribution of its vector species (*Culicoides sonorensis*), which in turn depends on soil and water characteristics.

Impact:

One of the mainstays of West Nile virus vector surveillance is the gravid trap, which works by attracting ovipositing female *Culex* mosquitoes to a reservoir of foul water brewed for the purpose. Artificial oviposition attractants developed by ARS would be more convenient and produce more consistent results than fermented organic suspensions.

The discovery of oviposition repellents opens an entirely new strategy for mosquito control. The repellents could be used to protect sensitive habitats or to herd mosquito populations toward toxicants.

The understanding of the ecology of bluetongue virus distribution is a powerful tool for development of large scale risk assessment, helping producers and extension target surveillance efforts.

Mosquitoes

Traps for gravid *Culex* mosquitoes, used for arbovirus surveillance, often rely on hay infusion as an attractant. This infusion requires replacement frequently and is not standard in attraction. New compounds were identified by GC/MS from different extracts of hay infusions. The design of gravid traps affected the number of *Culex quinquefasciatus* captured. Larger and darker pans received more egg rafts (Allan and Kline, 2004). Using olfactometer assays, several compounds were identified that attracted gravid *Culex* mosquitoes. These compounds can form the basis for development of controlled-release lures for gravid *Culex* traps. In the laboratory, dairy effluent was the most attractive for oviposition by *Culex quinquefasciatus.* However, an infusion of cow manure was most attractive for both *Culex quinquefasciatus* and *Culex nigripalpus* in the Florida fields (Allan, et al., 2005).

Culex tarsalis is an important vector of West Nile virus in the western United States. Wyoming has had an increasing problem with this species as a result of the proliferation

of coal bed methane ponds. Satellite imagery showed a 75% increase in these larval sites in the Powder River Basin from 1999-2004 (Zou, et al., 2006b). The virus was shown to be responsible for a significant decline in populations of the threatened sage grouse (Naugle, et al., 2004). It was shown that 12 compounds that act as topical repellents of biting mosquitoes were also repellent to ovipositing *Aedes albopictus* (Xue, et al., 2003, Xue, et al., 2006). A study intended to find chemicals that attract ovipositing females of this species instead showed that none of the compounds associated with attraction of biting mosquitoes (dimethyl disulfide, indole, 4-methylphenol, 3-methylindole, trimethylamine) were attractive to ovipositing mosquitoes. These chemicals also did not stimulate an electroantennogram (EAG) response (Trexler, et al., 2003).

Biting Midges
Working in British Columbia, Washington, New Mexico, and Illinois, ABADRL scientists showed a correlation between soil and water properties and occurrence of the three species of the *Culicoides variipennis* Group. *Culicoides occidentalis* was associated with the highest electrical conductivity and also with high calcium concentrations. *Culicoides sonorensis* (vector of vesicular stomatitis virus and bluetongue virus) was associated with high electrical conductivity and higher chloride concentration. *Culicoides variipennis* was associated with the lowest electrical conductivity levels (Schmidtmann, 2006).

1.1.2 Determine the dispersal patterns, breeding habits and host attractions of horn flies, house flies and stable flies that may be useful in devising control strategies. (Termites and fire ants added for this report).

Summary of Accomplishments:

- Genetic characteristics of individual cattle correlate to susceptibility to horn fly attack, providing new targets for genetic selection of livestock.
- Stable fly distribution depends on temperature history of the previous winter. Use of liquid manure creates stable fly habitats in pastures.
- House flies follow edges of habitats during dispersal and tend to concentrate in cul de sacs of favorable habitat.
- Though slow growing, the social structure of Formosan subterranean termite colonies supports good survival of adverse conditions (flooding) and the ability to quickly colonize new territory.
- *Reticulitermes* subterranean termites in France are probably the North American *R. flavipes*, suggesting that it is an invasive species in Europe.
- Fire ants in the U.S. have a different social structure from the majority of fire ants in their native South American range. Based on genetic studies, fire ants have probably been introduced into the U.S. multiple times, indicating that continued interdiction would be advisable.

Impact:
Cattle could be selected for resistance to horn fly attack, reducing production costs.

The use of liquid manure should be restricted or modified in order to avoid the production of stable flies.

Although not native to the U.S., Formosan subterranean termite populations are resilient. This indicates that human intervention is necessary to reduce the damage from this pest.

Some invasive pest insects act differently in their new ranges than they do in their native habitats. Control programs must be designed based on characteristics of the pest in its home region.

Horn Flies

The conceptual framework of using immunological interventions to protect animals from biting arthropods was reviewed as the introduction to a study of the cattle genetics of horn fly (*Haematobia irritans*) attraction (Pruett, 2002). A quantitative study of the association of horn flies with particular cattle individuals showed that some cattle are persistently less attractive than others, though even these "low carriers" exceed economic thresholds when fly populations are high. The suggestion was made that the basis of cattle resistance to horn flies might be the ability to coagulate blood normally in the presence of the salivary anticoagulant, thrombostatin (Pruett, et al., 2003). Subsequent work (Untalan, et al., 2006) showed that the genotype of the thrombostatin gene of horn flies varied regionally and, more significantly, varied between those flies infesting low-carrier cattle and those infesting high-carrier cattle.

The KBUSLIRL continues to accumulate a significant genomic DNA database on cattle that have been phenotyped for horn fly carrying capacity. The database should provide a fruitful biological asset for gene mining and subsequent publication. In 2006, an additional 24 calves were phenotyped for horn fly carrying capacity, and genomic DNA was isolated and archived for future gene mining studies bringing the database total to 79 cows and 140 calves. Most importantly, this database served as the focus of an NRI grant proposal that was submitted in June 2006 with Drs. Patricia Hollman and James Womack of Texas A&M University (College Station, Texas), entitled "Identification of Genes Associated with Tick Resistance or Susceptibility in Cattle", which will be resubmitted in 2007 following accumulation of more data.

Stable Flies

Models based upon temperature and precipitation, from a five-year study of seasonal dynamics of the stable fly (*Stomoxys calcitrans*) populations in eastern Nebraska, were developed to determine the effects of those variables on population levels and to predict population trends for control efforts. Three significant factors affected stable fly development: temperature, precipitation and nutrient application. Temperature was the most important factor in predicting stable fly population levels. Lower temperatures during the previous winter (November – February) were associated with higher population peaks (Broce, et al., 2005). Precipitation had an effect on the populations, especially during mid-summer. MLIRL observed that liquid manure was a significant attractant to an oviposition site. It is the product of feedlots that store effluent from cattle

and then spread it as"gravy" on fields, providing plant nutrients. Stable flies were strongly attracted to fields that had received applications of liquid manure.

House Flies
The hypothesis that house flies (*Musca domestica*) follow corridors of habitat conducive to dispersal was supported by a field study using marked flies in Georgia. The path was highly influenced by the nature of forest edges. One of the interesting aspects of this study was that flies tended to concentrate in areas that represented "cul de sacs" of habitat (Fried, et al., 2005).

1.1.3 Characterize the colonization behavior of Formosan termites and fire ants, and hybridization of invasive ants with native species.

Termites
The center of ARS research on Formosan subterranean termites (*Coptotermes formosanus*) is New Orleans. Hurricane Katrina was the occasion for a disturbing, though biologically interesting discovery. The New Orleans Mosquito and Termite Control Board collaborated with FSTRU to document that 20% of stumps in one area and 21% in another maintained viable colonies of the Formosan subterranean termite despite the flooding of New Orleans. Although the biological mechanism remains unknown, some of those colonies lived through conditions as harsh as submersion under brackish water, sometimes up to fifteen feet deep, and up to three weeks in duration.

The movement and extent of individual colonies was studied in a number of ways, taking advantage of molecular analysis of population genetics. Texas A&M University has documented the expansion of the Formosan subterranean termite into more Texas counties. The FSTRU, NOMTCB, and Louisiana State University showed that swarms consist of individuals from different colonies in a structure that appears to minimize the possibility of inbreeding (Husseneder, 2006). The University of Florida has shown that a separate species, *Coptotermes gestroi,* is expanding its range, raising the possibility of another damaging invasive termite in the United States. The geographic distribution of *Coptotermes formosanus* was reviewed in light of mitochondrial DNA studies. The study showed that the Formosan subterranean termite was probably introduced two different times into the United States (Austin, et al., 2006).

Studies of the biology and physiology of termites are another source of information that contributes to risk assessment. FSTRU and the University of Florida examined colony growth, finding that termite tunnels expand in width as a result of colony expansion, the need to remove dirt, and the constant maintenance of the walls of the tunnels. FSTRU also found that colony growth is proportional to the amount of food that is foraged. In another study, scientists focused on the composition of individual colonies of the Formosan subterranean termite for several years, then eliminated a number of colonies. In some cases, adjacent colonies expanded into the territory of the destroyed colonies, but in others, previously undetected colonies quickly expanded to fill the void. Studies of these colonies showed that genetically distant colonies were more aggressive toward each other than those genetically related. This observation was in contrast to a laboratory

study showing that aggression was related to the similarity of colonies' diets. Most colonies studied in Japan, North Carolina, South Carolina, and Louisiana were formed from single-mated pairs, though a smaller percentage (10-20%) were formed from groups of neotenic reproductives (Florane, et al., 2004, Husseneder, et al., 2005b, Messenger and Su, 2005, Vargo, et al., 2003, Vargo, et al., 2006). The bacterial flora of the termite gut was studied by identifying 16SrDNA and by culture, showing that over 30 species are present (Husseneder, et al., 2005a). A transformed bacteria (*Enterobacter cloacae*) spread throughout a colony when introduced on filter paper, raising the possibility of a "Trojan horse" strategy for control (Husseneder and Grace, 2005).

Termite social structure is complex and it responds to the various challenges experienced by the colony. During the last year, FSTRU and Louisiana State University found that a single colony can change from a single-mated pair (king and queen) to multiple pairs of reproductive termites. The colonies develop slowly, requiring three to seven years to reach their maximum population. The primary queen of a colony does not reach full maturity (physogastric) for seven years, at which time she lays about 100 eggs per day. In the absence of mature workers, nymphs were able to maintain the nutritional status of the colony (Crosland and Su, 2006b). Close examination of colonies also resulted in the first description of the sperm and spermatheca (female sperm storage organ) of the Formosan subterranean termite. Termites were shown to detect and orient toward food sources underground (Su, 2005).

A genetic study of *Reticulitermes* from Chile, France, and California clarified the systematics and movement of species within this genus. Using a series of mitochondrial and somatic DNA markers, it was shown that *Reticulitermes santonensis* of France is identical to North American *Reticulitermes flavipes*. This discovery has implications for termite control in France, but it also raises the possibility that new genetic developments in Europe could be introduced back into the native North American range (Su et al. 2006).

Fire Ants

ARS assembled data that contributed to a better understanding of how imported fire ants fit into North American habitats (http://www.msstate.edu/org/mississippientmuseum). Studies sponsored by the BCPRU produced a more precise picture of the potential damage caused by imported fire ants to our native ant species in the Southeast. They found that native ant composition, but not overall diversity, were affected. They also found that native *Reticulitermes* subterranean termites commonly inhabit fire ant mounds in Alabama (Shelton, et al., 2003). In Arkansas, the black imported fire ant (*Solenopsis richteri*) does not affect ground foraging native ants. Three species of native ant (*Monomorium minimum, Pheidole bicarinata,* and *Paratrechina vividula*) were actually positively correlated with the occurrence of the imported fire ant. Scientists observed how another species of exotic ant, *Pheidole obscurithorax,* attacks chicks. They also assessed the imported fire ant problem in important agricultural landscapes, finding that fire ants were most often associated with non-forested areas or forest clearings, non-diverse flora, and soil with higher silt content. The antennae and compound eyes of fire ants were studied, producing basic information that contributes to an understanding of

how the ants establish and expand their colonies. Ultrastructural studies were performed on the antennae and eyes of phorid flies that attack fire ants. On a genetic level, scientists collected 3,281 ants from 363 mounds and amplified microsatellite DNA, preparatory to determining the interrelationships of individual colonies. BCPRU found a genetic allele associated with colonies of red and black fire ant hybrids (*Solenopsis invicta x richteri*) that appear to be a marker for polygyne (multiple queens) colonies.

A climate-based model (Morrison, et al., 2004) was constructed by scientists at the IFAHIRU with the cooperation of the University of Arkansas at Monticello and the Institute of Global Climate and Ecology in Moscow. The model was designed to predict future range limits for the red imported fire ant, *Solenopsis invicta* in the United States and the world. Key parts of the model included the effects of temperature on colony growth, worker longevity, winter kill, and seasonal production of winged queens. The model was driven by daily maximum and minimum temperatures and fit to the current extreme northerly distribution of fire ants in Tennessee where winter kill appears to be limiting colony survival. After more than five years, the model's predictions still appear to be accurate. The model of potential fire ant range limits in the United States helps quarantine officials to better focus their efforts on areas where major expansion is still likely, especially in New Mexico, Arizona, California, Oregon, Virginia, and Maryland. The worldwide estimates of fire ant range limits have been very useful to policy makers in Australia, New Zealand, Taiwan, Hong Kong, and China because this information allows them to evaluate the geography and the magnitude of the threat posed by recent infestations of fire ants in their respective countries. Potential range estimates have also helped quarantine officials around the world to know which regions are susceptible to invasion.

BCPRU also assessed the imported fire ant problem in important agricultural landscapes, finding that fire ants were most associated with non-forested areas or forest clearings, non-diverse flora, and silt content of the soil. Ultrastructural studies were performed on the antennae and eyes of phorid flies that attack fire ants. On a genetic level, scientists collected 3,281 ants from 363 mounds and amplified microsatellite DNA, preparatory to determining the interrelationships of individual colonies. IFAHIRU developed a quick and effective PCR test to determine whether fire ant workers belong to polygyne (multiple queens) or monogyne (single queen) colonies (Valles and Porter, 2003). Fire ants apparently concentrate phosphorus in their colonies, as 2.13% of fecal dry weight consists of phosphoric acid. Even the larvae secrete phosphoric acid in their anal fluid (note that the hindgut is not connected to the larval rectum) (Chen, 2005b).

Funded by a National Research Initiative grant, the IFAHIRU studied the genetic structure of imported fire ant populations. Working with the University of Georgia, the University of Nebraska, and Rockefeller University, they used numerous genetic markers and recently developed analytical methods to reconstruct the invasion history and spread of fire ants in the United States. This groundbreaking study was the first to convincingly demonstrate that multiple introductions of fire ants into America have occurred and that the subsequent spread of fire ants involved natural dispersal as well as human-mediated dispersal (Shoemaker, et al., 2006).

The polygyne form of the red imported fire ant was thought to occur primarily in discrete populations embedded within areas composed of monogyne colonies. This distribution implies that polygyne colonies compete with monogyne colonies and subsequently create a population homogenous in social form. Polygyne colonies produce mostly sterile males, so opportunities for insemination of female alates may be minimal at the centers of large polygyne areas. To test for homogeneity in the distribution of each social form, a large defined area in north central Florida previously shown to consist of the polygyne social form was examined in finer detail for the presence of single queen colonies. A sample of 46 - 51 colonies from each of six sites located along an east-west transect through the middle of the polygyne area and a site at the northernmost limit of the polygyne area was taken. Several hundred workers from each nest were collected along with nest material. The social form of each sample was determined by an aggression test of workers to the introduction of non-nestmates and by the dissection of males for sterility. Both fire ant social forms were present at all collection sites and 103 of 333 (30.93%) colonies sampled were determined to be monogyne colonies. Among the collection sites, the percentage of colonies that were monogyne ranged from 3.9% to 57.4%. The polygyne region in north central Florida and probably elsewhere are most accurately described as a mosaic where relatively high frequencies of polygyne colonies are interdispersed with single queen colonies. This work alters the existing view of polygyne populations and emphasizes the inter-relationship of the two social forms (Fritz and Vander Meer, 2003). Monogyne colonies predominate in Mississippi (Vogt, et al., 2003c).

1.1.4 Perform and categorize genetic profiles and behavior of New World screwworm (*Cochliomyia hominivorax*) in South America and the Caribbean in support of potential eradication efforts by APHIS.

Summary of Accomplishments:

- Different populations of screwworm flies respond differently to sex pheromones in the cuticular hydrocarbons.

Impact:

New strains of screwworm fly must be tested for mating fitness with flies from the region to which they will be applied.

Screwworm Flies

Working with the University of Florida and the International Atomic Energy Agency, scientists at the MFRU showed that screwworm flies in Jamaica, Venezuela, the Dominican Republic, and Trinidad & Tobago did not have the sex pheromone expressed by Central American flies. Cuban flies only partially expressed the pheromone. The implications for sterile male release programs are not clear, though the absence of sufficient mating attraction would presumably hurt control efforts. These pheromones are part of the cuticular hydrocarbons of female screwworm flies, including 6-acetoxy-19-methylnonacosane (affects all strains) and 7-acetoxy-15-methylnonacosane (affects

one Panamanian and a Jamaican strain, but not a different Panamanian strain) (Mori, et al., 2004a, Mori, et al., 2004b, Carlson, et al., 2006, Carlson, et al., 2007).

1.2 Epidemiology

Summary of Accomplishments:

- More active medical intervention following envenomation from fire ants may be justified, based on specific effects on the immune system.
- Biting midges can cause significant cellular damage from the host reaction to the midge saliva.

Impact:
The bites and stings of biting midges and fire ants may be more damaging to the skin than previously thought, justifying more energetic efforts to prevent bites.

Biting Midges and Fire Ants: Toxicity
Toxicity of fire ant bites was studied by IFAHIRU and toxicity of *Culicoides* bites was studied by ABADRL. The unique fire ant venom contains mainly piperidine alkaloids that have a wide range of physiological activities. However, the often life-threatening allergic reactions to stings are attributed to a small amount of proteins found in the venom. While hypersensitivity to fire ant venom accounts for many of the reported medical problems associated with fire ants, there are reports of deaths and neurological problems after individuals receive large numbers of fire ant stings that are more readily explained by direct toxicity. Nitric oxide, a neurotransmitter, plays important roles in various physiological and inflammatory processes throughout the body. Scientists at IFAHIRU and the University of Mississippi Medical Center, Jackson, Mississippi, used a rat mammalian model to determine that fire ant venom alkaloids inhibit the production of nitric oxide, by several forms of nitric oxide synthase. The inhibition of nitric oxide production could cause the reported systemic neurotoxicity, hypercoagulability, or other systematic toxic effects in man after receiving massive stings, independent of the recognized protein-based hypersensitivity reactions. This study indicates that a more aggressive rather than passive approach to management of massive fire ant stings may be necessary (Yi, et al., 2003). The details of cellular damage from *Culicoides sonorensis* saliva were described in a guinea pig model (O'Toole, et al., 2003).

1.2.1 Determine the role of species biology and population genetics in the transmission of arboviruses.

Summary of Accomplishments:

- Quarantine regulations can be considerably modified based on the failure of northern populations of *Culicoides sonorensis* to transmit bluetongue virus. Models of transmission further specify locations likely to be susceptible to bluetongue virus.
- Vesicular stomatitis virus is transmitted vertically and horizontally by *Culicoides,* the saliva of which facilitates infection. There is evidence that North American

phlebotomine sand flies and grasshoppers also play a role in transmission to cattle.
- West Nile virus in the northeastern U.S. is transmitted by a variety of mosquito species. Transovarial transmission appears to be a significant source of virus amplification.
- Mathematical models of spatial distribution successfully mapped risk of West Nile virus in California and successfully predicted an epidemic of Rift Valley fever virus in East Africa.

Impact:
The discovery that northern tier states have biting midges incapable of transmitting bluetongue improves trade in live cattle.

Biting midges must be controlled in order to prevent transmission of vesicular stomatitis virus during outbreaks. Other insects may also be involved in transmission.

Mosquito control is important even in the absence of West Nile virus infected birds, because the virus is maintained transovarially between mosquito generations.

Mathematical models of mosquito-borne viruses can be used to save money for surveillance by targeting efforts toward the most likely transmission sites.

Biting Midges
Scientists at ABADRL showed that the strains of *Culicoides sonorensis* in Montana and Alberta (Canada) were not capable of transmitting blue tongue virus. The occurrence of blue tongue virus can cause considerable complication to the movement of animals, as well as direct damage to some species. This discovery changes the assessment of risk resulting from movement of infected animals into this region. This work was accomplished using the latest genetic techniques and with the cooperation of Agriculture Canada. Flies challenged in 2004 and 2005 were held for 12 days at 22^{o}C to test the effects of higher temperature on virogenesis. Flies were tested for infectious virus by cell culture, and the presence of BTV RNA by PCR. 10% of the colony flies were positive by cell culture, and approximately 70% positive by PCR. None of the Alberta midges were positive by cell culture, yet 88% were positive by PCR. 1.4% of the Montana specimens were positive by cell culture, with 72% positive by PCR. PCR positives represent the presence of BTV RNA and do not necessarily reflect infectious virus. The data available to date suggest that the populations from which the test flies were collected in Alberta and northern Montana are refractory (non-susceptible) to infection with bluetongue virus.

The 2004 and 2005 study confirmed previous data from 2003, and supports the reduced testing of Montana cattle for bluetongue virus before export to Alberta feedlots.
The laboratory also worked on another virus that is a problem for U.S. agriculture, vesicular stomatitis virus. In response to an unprecedented outbreak of the disease in Wyoming, scientists collected a wide variety of insects to determine whether more than black flies and *Culicoides* are capable of transmission. They found that grasshoppers (*Melanoplus sanguinipes*) became infected when they were fed infected cell culture

material, the bodies of dead, infected grasshoppers, or infected cattle saliva. One third of cattle acquired infection from grasshoppers when the grasshoppers were mixed with feed. (Nunamaker et al. 2003) The phlebotomine sand fly, *Lutzomyia apache*, may be another vector of VSV. Recent outbreaks of VSV in the western U.S. coincided with the distribution of the sand fly, based on ecological niche modeling of the species habitat (Schmidtmann et al. 2002, Herrero et al. 2004).

Research filled in many details of the role of *Culicoides* as vectors of VSV. First, it was shown by in situ hybridization and immunohistochemistry that infection progressed from the foregut and midgut all the way to the rectum by five days after inoculation. Neural, epithelial, and hemocyte tissue were all infected, though there was no damage to the host (Drolet, et al., 2005). The presence of *Culicoides* saliva enhances vertebrate infection by VSV. The active component may be a 66 kDa protein that downregulates the immune response of vertebrate cells (Bishop, et al., 2006). Horizontal transmission of VSV between flies through the vertebrate host has been difficult to demonstrate (Perez de Leon and Tabachnick, 2006), but recent work suggests that co-feeding (transmission of virus between simultaneously feeding flies) may be the mechanism (Perez de Leon, et al., 2006).

ABADRL is also working on complete genome sequencing of *Culicoides sonorensis* mitochondrial DNA. They have established a strategy for this large project that consists of two levels of replication, involving replicate individuals and replicate colonies of the insects. Completion of this work will provide a valuable tool for studies of population genetic structure of these vectors.

The distribution of bluetongue virus was studied in 110 herds of cattle in Nebraska, North Dakota, and South Dakota. Abundance of infection was positively correlated with altitude, the import of cattle from infected states, and the presence of *Culicoides sonorensis* (Green, et al., 2005). These studies resulted in production of a mathematical model of BTV infection of *Culicoides sonorensis* based on temperature and latitude (Mullens, et al., 2004).

Mosquitoes

A study by the MFRU in cooperation with the Connecticut Agricultural Experiment Station tested hundreds of thousands of mosquitoes to show that West Nile virus in that state is principally transmitted by *Culex pipiens*, particularly those located in the treetops. They also showed that *Culex restuans, Culex salinarius, Culiseta melanura,* and *Aedes vexans* are important vectors of the virus (Anderson, et al., 2004). Most recently, the Connecticut laboratory has demonstrated the importance of transovarial transmission of West Nile virus in *Culex pipiens.* They have been able to show in a laboratory model that a small percentage of non-bloodfed, overwintering progeny of infected mosquitoes emerge from diapause up to six months later able to transmit the virus. Transovarial transmission may be more than an overwintering mechanism for the virus. In late July and August, there is evidence that transovarial transmission amplifies the virus by spreading it to significantly more mosquitoes than would be the case were horizontal transmission the only mechanism.

The ABADRL and Suffolk County, New York, Health Department detected 38 West Nile virus positive pools from collections of 13,296 mosquitoes. In that location, *Culex pipiens* and *Culex restuans* were principal vectors. *Culex salinarius* was important when the virus became abundant later in the season. ABADRL also worked with the Wyoming Department of Health. There, *Culex tarsalis* was the most abundant mosquito (4,682 of 9,929 collected) and also the most commonly infected with West Nile virus (8 per 10,000 minimum infection rate). The Wyoming Department of Agriculture helped out with a research project performed by ABADRL designed to determine the host preference of the three most common mosquitoes in the state. *Culex tarsalis* fed on deer, cows, pronghorn antelopes, moose, humans, rabbits, voles, sparrows, pheasants, ducks, hawks, orioles, snipes, owls, and sage grouse. *Aedes vexans* fed on deer, moose, cattle, pronghorn antelopes, humans, voles, pheasant, and sparrows. *Aedes dorsalis* fed on deer, cattle, pronghorn antelope, moose, humans, and rabbits. These results imply that control of West Nile virus may depend on control of *Culex tarsalis*, because this species feeds on a wide variety of both birds and mammals. MFRU's work in the laboratory shows that lactic acid is less attractive to *Culex;* this may be an indication of the physiological basis for this host preference.

Another aspect of risk assessment is the development of mathematical or computer models. The MFRU worked with the National Aeronautics and Space Administration to develop a spatial model of the risk of Rift Valley fever virus transmission. The model is based on regional predictions of rainfall that might hatch floodwater mosquitoes to initiate the disease transmission cycle. It also uses the normalized difference vegetation index (NDVI) as a surrogate for rainfall. That model was proven to be dramatically successful when it predicted the outbreak of Rift Valley fever in Kenya two months before the disease broke out in December, 2006 (Anyamba, et al., 2006a, Anyamba, et al., 2006b, Chretien, et al., 2006). In Wyoming, ABADRL and the University of Wyoming developed a model of the risk of West Nile virus transmission based on temperature. They produced a risk map for the state and used the model to accurately predict areas of more concentrated transmission in a county of California. Due to its' simplicity, this model may prove to be valuable for prediction of risk from many mosquito-borne viruses (Zou, et al., 2006a).

ABADRL made extensive collections of mosquitoes in Kenya during 2005 and 2006 while working on Rift Valley fever virus in an attempt to understand its' relationship to vector species. They plan to examine gene flow between related species through a study of mitochondrial DNA. This basic work on population genetics will have practical results for risk assessment of potential vectors in the United States. It would be particularly interesting if a system of gene flow in African *Aedes* vectors is paralleled by a similar system in the unrelated *Aedes* taxa of North America. *Aedes mcintoshi* and *Aedes circumluteolus* samples that were brought back from Kenya have been matched to corresponding North American species and DNA has been extracted. In a small number of these, the mitochondrial gene, ND4, has been amplified and genetic variation has been detected using single strand conformation polymorphism (SSCP) analysis on acrylamide gels. Work will continue to PCR amplify ND4 from more samples and to complete SSCP analysis and DNA sequencing of different banding patterns. A protocol that uses

the ABI sequencer for fragment analysis is being developed to save time and achieve results more rapidly. More samples will soon be shipped from Kenya to complete collections from a wider range of locations to include areas hit by the recent RVF outbreak. Once these are paired with corresponding species, DNA will be extracted and processed as previously described. ABADRL has selected *Aedes triseriatus* transmission of LaCrosse encephalitis and West Nile virus as a parallel system in North America. Work is still in progress on comparing banding patterns and sequencing results.

1.2.2 Determine the role played by filth-breeding flies in the epidemiology of bacterial diseases in livestock and humans.

Summary of Accomplishments:

- House flies play a direct role in transmission of pathogens that are a direct threat to food safety.

House Flies

In a collaborative project with ARS research immunologist Peter Holt (Athens, Georgia), house flies became infected with *Salmonella enterica* within 24 hours of being released into a room with infected chickens. Dissection of flies revealed the presence of the pathogen on the surface and in the midgut but not in the crop or salivary glands. Healthy birds became infected by ingesting as few as five of these naturally-infected flies.

1.2.3 Determine the role ticks play in the transmission of Lyme disease in cattle and of other pathogens in animals and humans.

Summary of Accomplishments:

- Cattle probably act as significant reservoirs of *Borrelia burgdorferi*, though infection does not produce disease in cattle.
- Genetic diversity and host range of *Borrelia burgdorferi* and the habits of vector ticks have contributed to the rapid spread of Lyme disease.
- Gray squirrels and raccoons are reservoir hosts of the etiologic agent of human granulocytic ehrlichiosis.

Impact:

Efficient Lyme disease control may require tick control on cattle in order to limit the reservoir of the pathogen.

The U.S. should increase its vigilance against *Ixodes*-borne pathogens, because this group of ticks is biologically well-suited as a vector.

Wildlife interventions might reduce the prevalence of human granulocytic ehrlichiosis.

Ticks

Cattle exposed to *Borrelia burgdorfer*- infected *Ixodes scapularis* developed infections, with presence of the bacteria up to one year later in the thyroid. Some nymphs that fed

on infected cattle were able to transmit the infection to rabbits as adults. Cattle infection was not associated with any particular pathogenesis, and not with lameness.

An ARS contract with Yale University produced a series of research that described many aspects of pathogen transmission by ticks, with an emphasis on *Borrelia burgdorferi*. At a physiological level, they showed that impaired Toll-like receptor function enhanced transmission of the pathogen from mice to ticks, suggesting a mechanism for how some low virulence strains of *Borrelia* manage to maintain themselves (Bockenstedt, et al., 2006). The *Borrelia* apparently require a component of tick saliva for efficient infection, the experiment identifying the particular salivary protein involved (Rmamoorthi, et al., 2005). On a larger scale, correlations were drawn between regional climate regimes, host availability, and the ecology of *Borrelia*. This study attempted to explain the variety of species in different regions and makes the argument that *Borrelia* as a system of species has responded evolutionarily to local conditions (Kurtenbach, et al., 2006). In a similar theme, the generalist nature of *Borrelia burgdorferi* in the United States was related to its rapid spread (Hanincova, et al., 2006).

A computer model of dispersal of *Ixodes scapularis* suggested the following interesting hypotheses: 1) hosts with high tick burdens and large home ranges (e.g., deer) play a critical role in range expansion; 2) hosts with small home ranges (e.g., mice) can limit range expansion if they divert a sufficient number of ticks from feeding on more mobile hosts; and 3) birds that migrate annually (e.g., robins) can play a crucial role in tick range expansion (Madhav, et al., 2004). The case was made that *Ixodes scapularis* has not completed its distribution in the United States and that, based on climate, it will expand into new areas (Brownstein, et al., 2003) favored by habitat fragmentation (Brownstein, et al., 2005). These studies were used as a basis for quantitative estimates of the expansion (into the central U.S. and into Canada) and contraction (from the southern United States) of the range of *Ixodes scapularis* and Lyme disease risk in response to projected climate change (Brownstein, et al., 2005). Current risk was estimated nationally from actual survey data (Diuk-Wasser, et al., 2006).

Molecular studies of *Borrelia* showed that despite the division of European microflora into multiple species, there is even more variation within species, as determined by single-strand conformation polymorphism analysis capable of detecting single-base pair differences (Derdakova, et al., 2002). Double infections of mice with a pair of genospecies showed that one genospecies can dominate subsequent transmission to ticks (Derdakova, et al., 2004). Similar genetic diversity of *Borrelia burgdorferi* in the United States was documented (Bunikis, et al., 2004a). Finally, 16S-23S rDNA typing described the relatedness of relapsing fever agents (*B. miyamotoi, B. lonestari, B. turicatae,* and *B. hermsii*) (Bunikis, et al., 2004b).

The etiologic agent of human granulocytic ehrlichiosis (*Anaplasma phagocytophila*) was shown to be transmitted efficiently from gray squirrels and raccoons to ticks, suggesting that these common American mammals are important reservoirs (Levin, et al., 2002).

1.2.4 Determine the role played by screwworm in the transmission of foot-and-mouth disease and other viral pathogens of livestock.

Summary of Accomplishments:

- Screwworm flies have no role in the transmission of foot-and-mouth disease, nor in the transmission of African swine fever. This finding eliminates the risk of introducing these pathogens on new strains of screwworm flies used for the eradication program.

Impact:

Screwworm flies from Caribbean islands or South American countries may be used as sources of new strains for the production of sterile males, even though the source countries have foot-and-mouth disease or African swine fever.

Screwworm Flies

Screwworm flies were taken to Plum Island biocontainment facility in order to determine whether they could be involved in transmission of foot-and-mouth disease virus or African swine fever virus. This highest level of biocontainment was necessary because of the veterinary danger of these pathogens. Screwworm flies were not able to maintain infections. This work had been requested by the Animal Plant Health and Inspection Service (APHIS) because of the perceived risk of introducing these viruses with screwworm flies collected in the wild for establishment of new strains in the screwworm eradication program.

Component 2: Detection and Surveillance Technology

Prevention is better than cure. The harm caused by arthropods, whether as disease vectors or by direct damage, is proportional to their numbers. The earlier that a vector or pest is recognized, the greater the opportunity for effective suppression. One goal is to develop faster, cheaper more sensitive means to detect animal or human pathogens - both endemic and exotic - in vectors. Another goal is to detect and measure the arthropod itself. Finally, a system that combines detection methods with knowledge of arthropod biology can theoretically predict dangerous population surges from correlated environmental factors.

Detection and surveillance constitute the second of the four elements of Integrated Pest Management. Risk assessment recognizes and predicts the problem, but surveillance actually measures it. One of the challenges of surveillance is matching the qualities of the method to the actual operational needs. Sensitivity, specificity, cost, and scale are all considerations. For example, a method that collects many thousands of mosquitoes in order to detect a rare species may attain unnecessary sensitivity at unacceptable cost. A technique for initial detection of introduced Rift Valley fever virus in mosquitoes must have very good specificity in order to reduce the risk of a false alarm. Perhaps most important, the implications of the results of a single trap or a trapping grid are very much influenced by the scale of the problem examined. One trap in a community is representative of a more complicated situation, the nature of which may be quite

uncertain. The same considerations apply to effective management of fire ants and termites.

Component 2 Goals:

2.1 Detection and Diagnostics

2.1.1 Develop molecular or biochemical assays for the rapid detection of viruses and vertebrate parasites in Dipteran vectors and in ticks. Tests will be both sensitive and specific when using whole arthropods in pools of at least five. Also develop assays capable of detecting potential exotic invasives or indigenous recombinants (e.g., West Nile and St. Louis encephalitis viruses).

Summary of Accomplishments:

- Better diagnostic tests were developed for:
  - *Borrelia burgdorferi* (PCR)
  - *Boophilus microplus* (serology of cattle, genetic relatedness)
- Bluetongue virus (real time IR rtPCR, competitive ELISA, peptide nucleic acid probes)
- Epizootic hemorrhagic disease virus (real time IR rtPCR, competitive ELISA)
- West Nile virus (improved RNA extraction, improved column desorption)

Impact:
Risk assessment, surveillance, and diagnosis of the agents above have been improved.

Ticks
A PCR primer was developed that could be used to distinguish the etiologic agent of Lyme disease, *Borrelia burgdorferi*, from other species of *Borrelia* (Cyr, et al., 2005). A serologically defined 19.1 kDa protein was used to develop a diagnostic ELISA for cattle exposure to *B. microplus*. The 19.1 kDa protein has been partially amino acid-sequenced and we are attempting to isolate the gene. The information offers the potential for serological diagnosis of *B. microplus* exposure to cattle in support of the CFTEP program of APHIS-VS. In addition, it adds to a pool of candidate antigens for vaccine development.

Evaluation of the genetic relatedness of *Boophilus microplus* ticks collected from separate outbreak sites such as The Cattle Fever Tick Eradication Program (CFTEP) is a joint federal-state program designed to prevent *B. microplus* from reentering the United States. In support of this program we have provided technologies that will aid in elucidating the genetic relatedness of ticks collected from independent outbreaks of *B. microplus* ticks within and outside of the quarantine zone. This work lays the foundation for surveying genetic relationships between ticks collected throughout Mexico. The group fully sequenced the glucose-6-phosphate gene of *B. microplus* and evaluated nucleotide sequence variation of one of the introns in approximately 500 individual tick specimens collected from 15 outbreak sites within the quarantine zone. Nine intron

sequence variations have been identified, the frequencies of which distinguish collections from independent outbreaks.

Biting Midges

Bluetongue virus (BTV) infects beef and dairy cattle throughout the western half of the country. Although seldom serious, animals coming from disease-endemic areas cannot be exported, costing cattlemen millions annually. ARS scientists in Laramie, Wyoming, have compiled a DNA profile of all 24 recognized types of BTV in the world, only five of which are native to the United States. They developed and validated a rapid molecular test for all the types, as well as for a related virus, epizootic hemorrhagic disease virus, that often jumps from wild ungulates to domestic ones. Quantitative real-time DNA assays (real-time PCR) were also developed for the first time for vesicular stomatitis virus, which can be confused clinically with foot and mouth disease. This test is superior to earlier ones in detection of viral isolates from vertebrate and invertebrate hosts, using a single, endemic focus over time.

ABADRL developed a new technique for detection of bluetongue virus in *Culicoides* biting midges. The technique is based on reverse transcriptase (to translate the RNA of the virus to DNA) polymerase chain reaction (to amplify the target DNA), with detection by a chemical that fluoresces under infrared light. The advantage of the technique is that it achieves greater sensitivity by avoiding problems of background created by natural fluorescence under ultraviolet light. A test takes six hours to complete and can detect an incredibly low 0.5 plaque forming units per milliliter (Kato and Mayer, 2006). IR rtPCR detection is also being applied to the detection of dissemination of the virus within the vector. The same technique has been applied to detection of epidemic hemorrhagic disease virus (EHDV). Primers for IR-RT-PCR were designed, resulting in a highly specific EHDV assay. All eight of the known EHDV serotypes, and none of the closely related BTV serotypes, were detected. A different kind of test for BTV and EHDV detects antibodies in infected animals. Through genetic engineering, baculovirus strains were developed that express structural proteins from each of the related viruses. A competitive ELISA accurately distinguishes the two (Mecham and Wilson, 2004). In addition, a *Culicoides sonorensis* cell line was established for culture of BTV and EHDV (McHolland and Mecham, 2003).

The infrared based detection of PCR products described above improved sensitivity and efficiency of bluetongue virus testing. Current work is progressing on an even more sensitive technique that may eliminate the need for nucleic acid amplification. Development of a nucleic acid detection method using light-harvesting, conjugated polymers and peptide nucleic acid (PNA) probes is the basis for this progress. As little as 0.5nM single stranded RNA target can be detected. Optimization for increased sensitivity is ongoing. Solid phase attachment of the capture probe and the development of hybridization buffers and wash buffers are complete. These polymers are also being tested for enhancement of real-time PCR sensitivity, and other novel methods for virus detection, including probes without amplification.

Mosquitoes
ABADRL worked with Suffolk County, New York, Department of Health to double the sensitivity of PCR detection of West Nile virus by adding proteinase K to the extraction procedure. An entirely different method of detection used by the University of Wyoming School of Pharmacy used desorption ionization-mass spectroscopy to characterize proteins amino acid by amino acid. Adding "buckey balls" carbon (buckminsterfullerene) improved detection by increasing desorption from a surface.

2.1.2 Develop accurate, sensitive and non-destructive methods for detecting hidden populations of ants or termites using applied physics.

Summary of Accomplishments:

- Detection techniques for termites were developed and applied based on acoustics, infrared, chewing activity (breaking circuit in conductive sheet).
- Advanced statistics applied to mark-release-recapture and distribution models resulted in more accurate methods for evaluation of treatments and localization of termite colonies.
- Studies of spectral and thermal characteristics of fire ant mounds were applied to remote-sensing surveillance.

Impact:
There are better techniques for detection (and therefore treatment) of termites in trees and structures.

Application of insecticidal baits for fire ants can be targeted based on aerial surveillance.

Termites
Efficient termite control requires good surveillance methods, especially in the case of the Formosan subterranean termite that attacks buildings above the ground and also infests living trees. The FSTRU worked with the University of Mississippi to develop devices for acoustic detection. The approach systematically examined chewing and "head banging" as sources of sound, then successfully applied that knowledge to detection in living trees. The New Orleans Mosquito and Termite Control Board used acoustic and infrared detectors to locate, and then treat, infestations. The program moved toward more sophisticated acoustic signal processing, which could facilitate longer range detection. The New Orleans Mosquito and Termite Control Board surveyed all buildings belonging to the City of New Orleans, documented the presence of the Formosan subterranean termite in five additional parishes of Louisiana, and quantified the number of colonies (three) in Jackson Square of the French Quarter. An entirely new detection device was developed from plastic sheet sandwiching silver particles, providing a constant electronic monitoring of termite activity (Su, 2002).

Significant statistical models of termite activity provide the means of estimating populations from mark-release-recapture studies. The problem with the classic Lincoln Index for these studies is the large bias created collecting near the release site. The use of efficient marking techniques and sophisticated statistical models can be used to get more

accurate measurements of control and location of colonies (Crosland and Su, 2006a, Lee and Su, 2006, Lee, et al., 2006).

Fire Ants
Working with Alabama A&M University and an industrial partner, the BCPRU described the thermal signature of a fire ant mound, and then used that information to find infested areas. Full spectra satellite imagery was also fairly effective, detecting 47% of *Solenopsis invicta* and *richteri* mounds (Vogt, 2004). Basic background on the size of fire ant mounds in relation to habitat (Vogt and Oliver, 2006) contributes to the accuracy of remote-sensing approaches to surveillance. In another approach, the BCPRU collaborated with the University of Mississippi on studies of sounds produced by fire ants. They measured wing beat frequencies of alate ants (those that establish new colonies).

2.1.3 Design and test a model using GIS to direct fire ant control efforts.

Summary of Accomplishments:
- A combination of thermal imagery, spectral imagery, and mathematical modeling produced a system capable of detecting fire ant mounds from satellite imagery or aerial photography.

Impact:
Application of insecticidal baits for fire ants can be targeted based on aerial surveillance.

Fire Ants
The BCPRU worked on detection of fire ant mounds using aerial photographs and other remote-sensing technology. Multispectral data were capable of detecting 79% of fire ant mounds, in a refinement of technique published earlier (Vogt, 2004). They applied for two patents on point sensor technology and described the distribution of the ants in Christmas tree plantations.

The effects of rainfall, temperature, and maximum sun angle on imported fire ant mound shape characteristics (slope, height, and eccentricity) were quantified. In general, mound building activity was negatively correlated with maximum sun angle as long as temperature and rainfall were favorable. Increased mound building resulted in increased eccentricity in the plane of the ground, a characteristic useful for automated detection of mounds in aerial imagery.

2.1.4 Design and test models using GIS and remote sensing to direct screwworm countermeasures.

Summary of Accomplishments:
- A spatial gas diffusion model of screwworm movement produced a spatial model that successfully guided placement of a ground release station on Aruba, eradicating screwworm fly there.

Impact:
The screwworm fly was eradicated from the island of Aruba.

Screwworm Flies
Using a gas diffusion model of screwworm fly movement, the SRU made a model of probable distribution of the flies based on remote sensing data. The spatially-based model may eventually provide the information necessary to concentrate sterile male releases on areas that are most likely to have flies. Already, the model was applied to eradication of screwworm flies from the island of Aruba, where ground release stations were located in the most screwworm-susceptible areas (Phillips, et al., 2004).

2.2 Surveillance

2.2.1 Develop species specific traps that are light weight, inexpensive, low maintenance, and which are surrogates for individual human or livestock bait. Traps will preserve caught specimens for identification and pathogen detection for at least 12 hours. Traps will automatically register and transmit collection information.

Summary of Accomplishments:
- Carbon dioxide baited traps catch many more *Culicoides* when the extracts of the hair of certain mammals are included.
- Traps using specific attractants for certain species of mosquitoes could be combined with an automatic counting device to develop remotely operated traps.

Impact:
Appropriate attractants can be used to make vector-specific surveillance tools.

Biting Midges
A commercial trap baited with carbon dioxide and octenol was used to survey Scottish *Culicoides* on the Island of Skye. Adding hair extracts of some vertebrates increased the catch dramatically (water buffalo 262%, pony 40%, calf 20%) whereas others decreased the catch (red deer and sheep) (Mands, et al., 2004). The results suggest that specific attractants could be developed based on attractant and repellent odors from vertebrate hosts.

Mosquitoes
The MFRU worked through a series of detection technologies that count mosquitoes attracted to a trap. Although the design is still preliminary, a detection device based on light counts individual mosquitoes as they pass a sensor that costs about $100. Combined with specific attractants, this design might be a low-cost form of automated surveillance trap. A number of comparisons were performed to evaluate the efficiency of different kinds of traps. Dramatic differences were seen between wild populations of *Culex quinquefasciatus* and *Culex nigripalpus* in Florida, with the former species most abundantly collected by the Mosquito Magnet Pro and the latter species by the standard CDC trap with carbon dioxide (Kline, et al., 2006).

2.2.2 Identify and synthesize host specific attractants and adapt for use in traps or bait stations.

Summary of Accomplishments:

- Chemicals that attract mosquitoes to their hosts occur in complicated combinations that vary from individual to individual. Some compounds repel and others attract, forming a push-pull odorant cloud that defines host vs. non-host.
- The chemicals that attract house flies to objectionably odiferous baits can be combined into a much more acceptable, and equally effective, bait formulation.
- The alarm pheromone of fire ants was identified, with potential use for surveillance and control.

Impact:

New candidates for baits, repellents, and attracts were discovered.

New concepts in the sensory ecology of the orientation of mosquitoes to their hosts imply completely novel ideas for vector control.

Mosquitoes

The MFRU has performed extensive tests of human skin, blood, and chicken feathers in order to try to understand the universe of compounds that influence host selection by mosquitoes. In the laboratory, combinations of simple chemicals attracted a significant portion of a population of blood-seeking *Aedes aegypti*, even in the presence of a human hand. Pairs of chemicals that were particularly attractive (percentage attraction compared to human hand) were L-lactic acid and dichloromethane (80%), L-lactic acid and dimethyl disulfide (78%), and L-lactic acid and acetone (78%) (Bernier, et al., 2003).

The effect of chemical attractants varies with the population of mosquito tested. *Aedes aegypti* from Florida responded to as little as 0.03 μg/min of lactic acid, but populations from Brazil required 10.27 μg/min. The addition of caproic acid or ammonia also affected different populations differently (Williams, et al., 2006). The complexity of the interaction of attractant compounds and species of mosquitoes was documented in a study that showed that only *Aedes aegypti* was attracted to lactic acid mixed with acetone; only *Culex quinquefasciatus* was attracted to ammonia. The same study showed that a mixture of lactic acid, acetone, and carbon dioxide attracted as many *Aedes aegypti* as a human hand (Allan, et al., 2007).

Comparison of bovine and avian blood showed that blood has volatiles that influence host selection. *Aedes aegypti* landed preferentially on collagen casings filled with bovine blood whereas *Culex quinquefasciatus* and *Culex nigripalpus* landed preferentially on casings filled with avian blood (Allan, et al., 2006a). Feathers also have compounds that are differentially attractive to *Culex* mosquitoes (Allan, et al., 2006b). The proportions of compounds extracted from the surface of human skin by glass beads (dodecanethiol, 1-dodecanol, and 2-phenoxyethanol) were associated with whether or not an individual person was highly attractive to *Anopheles gambiae* mosquitoes (Galimard, et al., 2007). As we gather more information about the chemicals that define a certain host to a blood-

seeking mosquito, we should keep in mind that the individual mosquito is a dynamic system. A fascinating study showing that mosquitoes could be trained to seek artificial scents (Tomberlin, et al., 2006) suggests that, in nature, mosquitoes may be changing their preferences for certain compounds depending on previous associations with those compounds.

Marking mosquito larvae in such a way that the emerging adults are marked would be a very useful technique for studies of movement, population size, and longevity. Although radioactive substances work well, they are no longer acceptable environmentally.

Working with *Culex quinquefasciatus*, MFRU has continued the search for a vital stain that could be added to larval food. More development is going to be necessary, because the dyes tested to date affect developmental times and do not permanently mark the adults (Welch, et al., 2006).

House Flies
The MFRU developed new attractants for house flies based on the observation that 25% blackstrap molasses was as good as proprietary baits (Geden, 2005). The volatile (15 compounds) and non-volatile (45 compounds) components of molasses other than sugar were analyzed (Quinn, et al., 2007). Among these compounds were a mixture that is being patented as an attractant to house flies with the very practical property of having little odor detected by humans.

Fire Ants
The alarm pheromones of the red imported fire ant were found to be species-specific, possibly providing a tool for surveillance or control. An alarm pheromone component of the red imported fire ant was isolated and identified by IFAHIRU scientists. This compound is readily available, and is also attractive to fire ant workers. Incorporation of this component into a trap system will provide a unique surveillance tool for monitoring interstate commerce and to help detect fire ants in low-density situations, e.g. Australian eradication program. In addition, the fire ant alarm pheromone was species-specific and is a good candidate for incorporation into toxic baits leading to enhanced fire ant bait discovery, more consistent control, and fewer effects on non-target native ants. Studies of the sensillae of both larval and adult fire ants may also lead to development of new ways to repel and attract ants.

2.2.3. Design and test a model using geographic information system (GIS) technology and remote sensing to predict the ideal placement of traps for vector and fly surveillance. The model will determine the optimum number of traps and how they should be redeployed based on collections as the season progresses.

Summary of Accomplishments:

- Spatial distribution of reinfestation by the *Boophilus* tick vector of Texas cattle fever indicates that the program to exclude this serious pest is threatened.

- Models of mosquito distribution have been used to predict West Nile virus, Rift Valley fever virus, and malaria. Work on a small and large scale will rationalize the design of detection systems for invasive mosquitoes.

Impact:
Formal processes have been initiated to increase the level of research on prevention of Texas cattle fever tick on wild ungulates.

The epidemic of Rift Valley fever in Kenya was detected early (19 December 2006) because teams were placed in the field based on the ARS model.

Ticks
Data input into the GIS database of historical and current *Boophilus* infestations continues to be accomplished at a rapid rate by KBUSLIRL. Activities include traveling to Zapata County to copy data sheets, digitizing and producing GIS overlays of all known quarantined premises in Zapata County, receiving and inputting updated outbreak information to produce overlays and develop epidemiological models, preparing sequence maps of current infestations to analyze rates and spatial associations of outbreaks, working with APHIS personnel to develop GIS script that will aid them in geo-referencing and reporting quarantined premises, and working with APHIS on database development and remote sensing analyses.

Mosquitoes
The MFRU has approached this problem on a fine scale and on a large scale. The fine scale effort has involved trials in the field of various mosquito traps, and comparing the results to other measurements of the populations. The intended product is an accurate error term for trapping based on the density and habitat placement of the traps. The large scale effort involves modeling of the distribution of the mosquitoes of the United States based on data from mosquito abatement districts, the military and other sources. The data has been assembled and methods applied for modeling distribution. One of the attractive features of this effort is that it will produce a spatial error term for the estimated distributions.

Remote sensing techniques were applied to evaluation of habitats for the presence of malaria vector species in Korea. The study showed that *Anopheles sinensis* larvae were most abundant in rice fields in one area (Gangwha Island) and in areas with little vegetation in another (Paju County). In each case, the larvae were concentrated in a relatively small portion of the total land area, quantifying a non-random and clumpy distribution. These features could be detected and quantified in satellite images (Sithiprasasna, et al., 2005).

Component 3: Biology and Physiology
Advances in technology, especially in functional genomics (the cloning of genes expressed only during certain physiological or immunological states) have opened opportunities to engineer genetic and chemical methods to control harmful arthropods with little or no spillover into the environment or danger to beneficial species. Such

strategies must start with a comprehensive grasp of the biology being targeted. For example, identification of carrier peptides activated at odor receptors in mosquitoes could lead to the design of chemical repellents that will inhibit blood feeding, but must be founded on a detailed understanding of host seeking behavior and sense organ ultrastructure to succeed. It would make little sense to study receptors in the antennae if the crucial recognition is done at sites in the legs. Similarly, isolation of receptors on tick or midge guts to parasites or viruses could lead to veterinary vaccines or blocking drugs, but an intimate knowledge of the interaction of pathogen and vector is needed first.

Where necessary, research units will need to strengthen their capabilities in molecular biology, invertebrate pathology and physiology, and must recruit skilled personnel to take advantage of the new technology.

Component 3 Goals:

3.1 Genomics and Host-Pathogen Interaction

3.1.1 Use ultrastructural methods, as well as expressed sequence tag (EST) and bacterial artificial chromosome (BAC) libraries, to investigate the basis of tick digestion, salivation, egg production and susceptibility to protozoan parasites of livestock. Identify and clone genes crucial to these processes and design agents that will inhibit or disrupt their actions.

Summary of Accomplishments:

- The feeding system of ticks has been studied to produce a picture of the salivary and pumping systems.
- Genetic detection of organophosphate resistance was developed.
- Genomic studies of *Boophilus* have produced targets for vaccine development.

Impact:

Acaricide resistance testing has been improved as a part of routine testing of *Boophilus* ticks captured in the United States.

An international consortium to develop *Boophilus* genomics was funded.

Ticks

Characterization of molecules that are essential for tick feeding is one approach to the identification of targets for alternative novel control, specifically molecules produced in the salivary gland tissue of fed adult females that are potentially introduced to the bovine host via tick saliva. We have identified transcripts encoding five zinc metalloproteases and four kunitz-like proteins from salivary gland tissue of adult female ticks that had fed on the host for either 1-2 days or 4-5 days. In other ticks, the zinc metalloproteases are believed to have a fibrinolytic role and inhibit host angiogenesis (prevent wound healing) while kunitz-like proteins have been implicated in the inhibition of the host blood coagulation system. The immunome database was extended with the identification of 13 *B. microplus* larval proteins, 15 proteins from adult female salivary glands, and 6 proteins

from nymphs that elicit bovine host antibody upon natural tick exposure. Functional characterization of these molecules with respect to their role in successful tick feeding of *Boophilus microplus* will lay the foundation for their use as potential targets for alternative control technologies.

A greater understanding of the tick nervous system contributes toward the practical goals of managing acaricide resistance and also developing new acaricides. KBUSLIRL described the full genetic sequence of the operon of an essential respiration enzyme, G6PD, in one-host ticks. A 125 kb BAC clone that contains the gene encoding CzEST9 has been completely sequenced. Screening of the BAC library for clones containing the coding region for a CYP41-like organophosphate resistance-associated cytochrome P450 gene has begun. Screening of SAGE (Serial Analysis of Gene Expression) libraries found this P450 gene was over expressed in the San Roman strain upon exposure to moderate levels of coumaphos and is more abundant in untreated San Roman compared to the Munoz organophosphate susceptible strain.

They also used a baculovirus to express *B. microplus* acetyl cholinesterase, the first time this has been done. Knowledge of the wild-type molecule allows for the search for mutations that may account for resistance to organophosphates. An organophosphate acaricide is used in the import dipping vats maintained by the Cattle Fever Tick Eradication Program on the border with Mexico. This has resulted in concerns regarding the development of resistance to organophosphates, necessitating the need to understand the specific mechanisms of acetyl cholinesterase target-site insensitivity. In related work, neural acetyl cholinesterase extraction was developed for the diagnosis of insensitivity and the genotyping of the individual tick. This technology will find utility in the CFTEP conducted by APHIS-VS, as it allows for diagnosis as well as the screening of additional organophosphate compounds. The genotype frequency can be monitored within the tick population.

An anti-tick vaccine to protect cattle from one-host ticks is an attractive research goal, especially if the vaccine required only one dose and was long-lasting. Unlike most biting arthropods, ticks remain attached to the host for several days and therefore must deal with the full vigor of the host's immune system. Natural occurrence of tick immunity and the availability of a partially effective product are further encouragement that good tick vaccine can be developed. The current strategy at KBUSLIRL is to find a vaccine candidate by studying the genome of the tick. Much of the work is toward sequencing the entire *Boophilus microplus* genome in collaboration with a number of partner institutions. Throughout this year, the laboratory created 6,000 expressed sequence tags (used to identify expressed genes) in the midgut and ovary of *Babesia*-infected larval ticks. They also identified a 19.1 kDa immunogen injected by ticks and nine salivary gland components involved in prevention of wound healing and prevention of coagulation. The KBUSLIRL collaborated with The Institute for Genetic Research, Purdue University, and Washington State University to sequence clones and expressed gene sequences associated with *Babesia* infection of ticks.

The ovarial proteome of *B. microplus* has been analyzed by 2-D PAGE, focusing on *Babesia bovis* infection-induced differences. Specific proteins that are differentially expressed have been identified, extracted and submitted for initial mass spec analysis at the Protein Chemistry Laboratory, University of Texas Health Science Center in San Antonio, Texas. Functional characterization of these molecules with respect to their role in pathogen transmission will lay the foundation for their use as potentials targets for alternative control technologies of the vector and the pathogen.

3.1.2 Help organize and participate in an international consortium to sequence the entire genome of the tick *Boophilus microplus* and manage the bioinformatics to allow comparison of homologous genes in different genera.

Summary of Accomplishments:

- Organization, collaboration, and some funding have been arranged for the complete genomic sequencing of *Boophilus microplus*.

Impact:
An international consortium to develop *Boophilus* genomics was funded.

Ticks
Considerable work on sequencing has been accomplished in the course of projects with specific goals described elsewhere in this section. An EST (expressed sequence tag) library is being analyzed. Bioinformatic analysis of these ESTs is ongoing, including a gene ontology analysis of the updated *B. microplus* Gene Index Version 2 with plans to focus upon *B. microplus* putative G-protein coupled receptors.

ARS scientists at the Knipling-Bushland U. S. Livestock Insects Research Laboratory (KBUSLIRL) and the Cattle Tick Fever Research Laboratory (CFTRL) in collaboration with scientists at The Institute for Genomic Research (TIGR) and the Center for Disease Control (CDC) were able to accomplish the following goals:

1) Cot (concentration of ssDNA x time to reassociation) analysis quantifying portions of genome that are highly repetitive, moderately repetitive, and not repetitive.
2) BAC Library Synthesis
3) BAC Library End Sequencing
4) BAC Clone Screening
5) EST Sequencing
6) EST Annotation and gene index

This newly acquired knowledge and compilation of genomic resources has led to the completion of a white paper entitled "Proposal for the sequencing of a new target genome, the southern cattle tick, Boophilus microplus," F. D. Guerrero, V. M. Nene, J. E. George, S. C. Barker, and P. Willadsen, describing the resources, rationale, needs, and scientific support for a B. microplus genome sequencing project. The completed white paper was submitted to funding agencies (NHGRI and NIAID-NIH) in FY 2005. Submission of the white paper represents a major accomplishment in the first year of this

research project, and it represents the initial and essential event in securing adequate funding for the conduct of a sequencing project. Accomplishment of these goals has led to International Research with the Department of Primary Industries and Fisheries, Brisbane, QLD, Australia. The project is funded at $1,394,000.00 by the Smart State Innovation Projects Fund, National and International Research Alliances Program, and entitled "A Cattle Tick Vaccine for Tropical and Subtropical Beef Industries." The ever-increasing genomic and proteomic databases that are originating from this research project, and are being made available to investigators worldwide, should substantially impact the rational design of future strategies for the control of ticks and the pathogens they transmit.

3.1.3 Use differential expression and other methods to isolate and characterize membrane receptors, immune response, and other factors crucial to susceptibility of Diptera to vertebrate pathogens. Identify and clone genes crucial to these processes and design agents that will inhibit or disrupt their actions.

Summary of Accomplishments:

- Many aspects of the genetic structure of *Culicoides* were described, including gene expression during viral infection, linkage maps, mitochondrial DNA, and genes responsible for salivary enzymes. Correlations were drawn between viral susceptibility and some genetic sequences and bacterial flora of the gut.
- The firm association of vesicular stomatitis virus (VSV) with *Culicoides* was established through genetic studies. Research also showed experimental association of VSV with grasshoppers and statistical association of VSV with North American phlebotomine sand flies.

Impact:

The possibility of a vaccine against *Culicoides* transmitted veterinary pathogens based on midge salivary antigens was established.

Periodic outbreaks of VSV in the United States are associated with *Culicoides* biting midges, and possibly with grasshoppers and phlebotomine sand flies.

Biting Midges

Research that will result in better control of *Culicoides* proceeded at the ABADRL on two fronts. First, using cutting edge genetic techniques, scientists created linkage maps of the genome of *Culicoides sonorensis*, redesigned mitochondrial DNA primers to match those of other flies, and created an expressed sequence tag (EST) library of salivary and midgut cells. This work could lead to more precisely targeted toxicants and to anti-midge vaccines.

ABADRL field and laboratory research incriminated the biting midge, *Culicoides sonorensis*, as the primary, proven vector of bluetongue viruses in the United States. Data developed by the ABADRL also established that the northeastern part of America is bluetongue-free because it is outside the range of *C. sonorensis*. An ABADRL extensive investigation of the population genetics of the *C. variipennis* complex in North America

resulted in a taxonomic revision of the species complex, in which the three subspecies were elevated to species status. In a follow-up study, it was demonstrated that levels and types of dissolved salts in larval habitats differ among aquatic habitats occupied by respective species. In a study to develop methods for assessing bluetongue disease-free regions in support of export sales for U.S. livestock, 74 farms and ranches across Nebraska, South and North Dakota were sampled for *C. sonorensis*, the primary vector of bluetongue viruses in the United States. The results show that the presence of *C. sonorensis* populations is correlated with geographic regions where evaporation exceeds precipitation and soils are non-glaciated. This information will help to clarify the epidemiology of bluetongue in both enzootic and bluetongue-free regions, further illustrating that the presence of the vector *C. sonorensis* is a primary risk factor for the exposure of livestock to bluetongue viruses. These findings open the way for analysis of environmental factors that support the presence of *C. sonorensis* populations, as well as large scale mapping and prediction of *C. sonorensis*, using computer-based GIS methods. The demonstration of bluetongue-free regions of the United States has resulted in a relaxation of export regulations, thus improving the competitiveness of U.S. livestock and livestock germplasm in world markets.

The role of *C. sonorensis* as a vector of vesicular stomatitis virus (VSV) has been examined in the laboratory using experimental procedures, such as immunohistochemical techniques, which show that VSV passes through the midgut barrier, and thus infected insects may be a competent biologic vector. VSV in the salivary glands further indicates that *C. sonorensis* is able to vector VSV during feeding. The presence of VSV in the eggs suggests that VSV may transfer vertically from females to their offspring. A protocol has been developed to isolate VSV RNA from paraffin embedded insects which can then be used for PCR analysis; this will enable confirmation of viral replication within insect tissues where virus is no longer viable. Nucleocapsid mRNA was successfully amplified from paraffin embedded *Culicoides* by RT-PCR. This confirmed VSV replication in the virus fed insects. An extensive time course in vivo analysis of VSV infection in the biting midge, *Culicoides sonorensis,* showed that following ingestion, VSV replicated in salivary glands, ovarial tissues, and was excreted. This evidence may suggest bite, vertical, and mechanical VSV transmission may occur in *Culicoides sonorensis*. *Culicoides sonorensis* was shown to be a biologically competent vector for vesicular stomatitis virus (VSV). Virus passed efficiently through the midgut and salivary gland barriers and replicated throughout the insect. Virus replication and production were detected in salivary glands from days 3-13 post feeding and in the eggs from days 5-13. This is the first report of the vector competence of *Culicoides* for VSV. This body of evidence seems to convincingly demonstrate that *Culicoides* can be a vector of VSV, a point that has sometimes been controversial.

The ABADRL insect vector genomics program has identified genes for which transcription increases during virus infection. In addition, midgut and salivary gland cDNA libraries were prepared and sequenced. This information has been released to international databases and will allow the development of new hypotheses of vector capacity, as well as models to predict and understand the epidemiology of these diseases (Campbell and Wilson, 2002, Campbell, et al, 2005). Research that utilizes the genetic

markers and cloned genes produced by the ABADRL will increase our understanding at the molecular level of the vector competence of *C. sonorensis* for bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV).

Genes that could be related to BTV and EHDV vector competence were identified and efforts were concentrated on evaluating the expression of these genes in two insect colonies with high and low susceptibility to infection. This information has provided a foundation for understanding the genetics of *C. sonorensis* vector competence.

Culicoides sonorensis gene expression following infection with EHDV was also studied. Of the cDNAs identified in midguts at two days post inoculation, three encode translational machinery components, and three encode components that affect cellular structural features. Of the differentially expressed salivary gland cDNAs, only one was homologous to a previously identified gene, a putative odorant binding protein (Campbell, et al., 2004b). Scientists created linkage maps of the genome of *Culicoides sonorensis* and redesigned mitochondrial DNA primers to match those of other flies.

An EST database of genes expressed in the salivary glands and midguts of *C. sonorensis* has been published and is available through GenBank. Salivary glands and midguts express genes that could be related to vector competence and this information is being used in current research of a related project. The results of this research provide a foundation for research to identify vector competence genes, as well as new strategies for targeted control of arboviral disease transmission systems. The ABADRL has received two requests for international collaborations as a result of this work.

An initial study comparing sequence variation and gene expression of suspected vector competence genes in *Culicoides*-competent and not-competent in BTV transmission was completed. Transcript levels of four genes, that preliminary studies indicated may play a role in orbivirus susceptibility and resistance, were in all life stages of the two colony populations that were evaluated. This study provided evidence for intersection between the highly regulated LAR and FZ2 integrin-mediated cell signaling pathways. Further characterization of the translation products and gene silencing experiments could better define these complex interactions.

A method for obtaining significant quantities of *Culicoides sonorensis* secreted saliva has been developed. This will allow salivary protein characterizations never possible before, due to insufficient material for analysis.

Culicoides sonorensis linkage map. *Culicoides sonorensis* is the primary vector of bluetongue virus in the western United States. The availability of a linkage map for this species would provide an important tool for better understanding its genetic make up and potential targets for vector control. Additionally, it would provide the groundwork for mapping genes/gene groups that contribute to vector competence phenotypes. To date, F1 intercross families have been created that represent all possible combinations of males and females from the three *Culicoides sonorensis* colonies that are currently on hand at the ABADRL. Several of these have been maintained through multiple generations as far

as the F8 generation. Families have enough individuals to provide statistical significance to linkage analysis. They are ready to be used for linkage mapping. Primers specific to the *Culicoides sonorensis* genome have been designed from the EST database developed by ABADRL. Intercross families were created and are ready for use in SSCP analysis and linkage mapping, and primers have been designed for use in linkage mapping within fiscal year 2007.

Culicoides sonorensis mitochondrial genome sequencing. Because of difficulties in getting good PCR amplification and sequencing, primers have been redesigned to more closely resemble other Dipteran mitochondrial sequences. This will allow for more rapid and reliable results.

A study of *Culicoides* gut microflora may have provided some insight into the pathogen environment encountered prior to vector infection. Using 16S rDNA sequence analysis, 36 genera of bacteria were identified in the guts of two *C. sonorensis* groups (one colony, one wild) and one *C. variipennis* colony. All three had distinct gut microflora. This result is an indication that one of the factors that could possibly influence infection is the make-up of the gut microflora (Campbell, et al., 2004a).

Phlebotomine Sand Flies and Grasshoppers
Recent ABADRL research identified a non-hematophagous insect (grasshopper) as a potential source of infection and maintenance reservoir of VSV. This study demonstrated that VSV replicates in grasshoppers and can spread between grasshoppers and cattle, which may lead to a better understanding of the epidemiology of sporadic outbreaks of VSV among western livestock. Analysis of the distribution of the sand fly, *Lutzomyia apache*, in the mid-Rio Grande River Valley, New Mexico, using geographic information system methods indicates a potential role for this sand fly in the epidemiology of VS viruses in the southwestern United States. Samples of grasshoppers active on 10 livestock premises in Wyoming and Montana, immediately following confirmation of VS-NJ virus in horses, were collected for laboratory assay of possible infection. VSV-NJ diagnostic base-pair sequences have been obtained, but these specimens have yet to be tested due to the unavailability of BSL-3 laboratory facilities required for work with this pathogen.

3.1.4 Identify food animal genotypes naturally resistant to blood feeding arthropods, characterize the mechanisms, and isolate the responsible genes.

Summary of Accomplishments:

- Bovine genetic markers were identified for prediction of susceptibility to tick bite.
- A serological method was developed for evaluation of tick exposure.

Impact:
Cattle herds can be evaluated for risk of exposure to *Boophilus* and other ticks.

Ticks

In an effort to evaluate the role that the host immune system plays in acquired resistance to tick infestation, three bovine genetic markers located within the bovine leukocyte antigen (BoLA) complex were evaluated, using a genomic DNA database of calves phenotyped for tick-resistance/susceptibility to the Lone Star tick, *A. americanum*. Statistically significant associations were identified between two of the genetic markers and the tick-resistant phenotype, suggesting that genes in these regions may play a role in the manifestation of acquired resistance to tick infestation. The region analyzed is of interest, as it encodes genes responsible for the presentation of pathogen-derived antigens to the bovine immune system. Characterization of these gene sequences will enable the identification of genetic markers that can be utilized in a genetic marker-assisted selective breeding program to either include calves resistant to tick infestation or exclude calves susceptible to tick infestation. In addition, knowledge of these bovine immune response genes allows us to evaluate more specifically the bovine immune response to selected *B. microplus* candidate vaccine antigens.

DRB3 is a class IIa gene of the bovine leukocyte antigen complex (BoLA), the second exon of which is highly polymorphic since it encodes the antigen recognition site of the DR class II molecule. Analysis of the second exon of the *DRB3* gene from the phenotyped calves in our herd revealed a significant association between the *DRB3* 4401* allele and the tick resistant phenotype. This information provides a potential genetic marker for tick resistance in cattle.

Life-stage-specific *B. microplus* proteins that putatively elicit antibodies in cattle as a result of natural exposure were identified. A serologically defined 19.1 kDa protein was used to develop a diagnostic ELISA for cattle exposure to *B. microplus*. The 19.1 kDa protein has been partially amino acid sequenced, and we are attempting to isolate the gene. The information offers the potential for serological diagnosis of B. microplus exposure to cattle in support of the CFTEP program of APHIS-VS.

3.1.5 Use functional genomics and other methods to investigate the social behavior and susceptibility to biological control agents of fire ants and Formosan termites. Identify and clone genes responsible for pheromone production, detoxification enzymes and other metabolic products useful in developing new forms of control.

Summary of Accomplishments:

- DNA detection techniques were established for fungal parasites of termites, various pathogens of fire ants, and social structure of fire ants.
- Termite tissue was genetically transformed, proof of concept that genetic manipulation of termites is a possibility.
- Gene expression was examined following pathogen infection in various vectors, providing potential for genetic manipulation of vectors.
- Progress was made on establishing genetic linkage maps of the screwworm fly, an important step in the development of a male-only strain.
- A gene controlling apoptosis in mosquitoes was found, providing a valuable new mode of action for toxicants.

Impact:
New pathogens in termites and fire ants were discovered.

A new class of potential insecticides was discovered.

Essential steps were made in the creation of a male-only strain of screwworm fly, an advancement that would save APHIS approximately $4 million per year in production costs.

Termites
Working with Mississippi State University, the FSTRU developed some basic tools for the study and surveillance of fungal pathogens of termites. It is important to be able to identify disease causing fungi that affect termites in order to determine their usefullness as biological control agents, as well as whether they are naturally found in the region in which they may be used. DNA was extracted from various isolates of the insect pathogens *Metarhizium anisopliae, Metarhizium flavoviride* and *Beauveria bassiana*. The DNA was amplified via polymerase chain reaction using a universal fungal primer pair. The sequence of the resulting amplified DNA was determined in order to develop specific DNA amplification primers found useful in determining the species of pathogen that might be affecting diseased termites. Two different software packages were used to design these species-specific primers based upon the determined sequences and they were commercially made. When these primers were used to amplify DNA extracted from known fungal pathogens, several of them were too specific and recognized only a single strain of a fungal species. Other primer pairs were not specific enough, and DNA isolated from other fungal species cross reacted and were also amplified; therefore, these primers could not be used to identify the pathogenic strain. Work was conducted to change the polymerase chain reaction conditions in order to improve this specificity, but, to date, has not been optimized in order to enable such species differentiation.

Although genetic control of termites may be a long way off, a first step was taken in a cooperative project with Tulane University. Embryonic tissues of native subterranean termites (*Reticulitermes* spp.) and the Formosan subterranean termite, (*Coptotermes formosanus*) were successfully transformed using a vesicular stomatitis virus that expresses a glowing green protein. This demonstrates the feasibility of transforming termite tissues. Transformation of termite tissues with immortalization genes might allow the development of useful cell lines in order to assay for termite-associated viruses. Portions of region-causing protein (actin) expression from *C. formosanus* were cloned and the deoxyribonucleic acid (DNA) sequence that was determined proved to be very similar to the actin promoter of other insect species. Full length cloning of this actin promoter will provide the basis for improved transformation and expression of desired genes in *C. formosanus* tissue cultures.

Mosquitoes
Other research produced valuable background on mosquito physiology and behavior that might eventually be applied to control. ABADRL, with the help of Colorado State

University, applied gene silencing techniques to an *Aedes aegypti* and Sindbis virus model system, with plans to study *Culicoides* and bluetongue virus. This technique will help identify the role of various mosquito genes in the infection process. MFRU identified the gene responsible for regulation of programmed cell death (apoptosis), with obvious application to manipulation for mosquito control. They also completed the sequence of a mosquito iridescent virus, identifying 126 genes and showing that 27 of them were homologues of other iridoviruses.

Screwworm Flies
SRU scientists worked with collaborators at the University of Nebraska to establish elements of a screwworm genetic linkage map based on AFLP markers. The linkage maps will facilitate a new research program to create a strain of screwworm flies that only produces males.

Fire Ants
Scientists from IFAHIRU and the University of Georgia completed a study describing naturally occurring molecular DNA sequence variation at the single gene *Gp-9* in fire ants. Extensive analyses of these DNA sequence data showed an important variation in fire ant social behavior. The tolerance of multiple queens in a single colony can be attributed to two critical amino acid changes within a single protein. This study yielded information of use in bridging population genetic and functional approaches to understanding the genetic basis of an important social trait. The trait was associated with genetic markers that could be determined through a PCR analysis.

They also identified the transferrin gene cDNA, gene architecture, and response to fungal infection from the red imported fire ant. They determined that the gene was induced up to 7-fold upon infection with the fungus, *Beauveria bassiana*. Genes, like *Solenopsis invicta* transferrin (SiTf), responding to microbe attack or infection, may provide a unique approach to assist in the discovery of microbial control organisms for the target insect pest. Future studies of the biochemical pathways in which the *Gp-9* product functions, as well as the phenotypic effects of molecular variation at *Gp-9* and other pathway genes, will lead to significant progress toward understanding the evolution of this key social adaptation.

Molecular biology and genetic techniques are important tools for use in the discovery, detection, and development of new biologically-based methods of control. ARS scientists from IFAHIRU developed a molecular-based assay for detecting two Microsporidian pathogens of fire ants. One rationale for developing this molecular assay was that the current screening methods (microscopy) are extremely time consuming, labor intensive, and incapable of detecting vegetative stages of these organisms. The new PCR-based method provides a means to efficiently screen large numbers of fire ant colonies for the presence of the pathogen, as well as provide the opportunity to identify intermediate hosts of this microorganism that may facilitate the spread the disease to uninfected ants (Valles, et al., 2002, Valles, et al., 2004).

Scientists from the IFAHIRU also discovered a protozoan pathogen (*Mattesia* sp.) that causes yellow head disease in fire ants and a fungal pathogen attacking fire ants (*Myrmicinosporidium durum*). Discovery of these pathogens is significant because both represent potential bioinsecticides of fire ants. Their discovery and characterization may facilitate discovery of similar pathogens in South America that may serve as classical or self-sustaining biological control agents (Pereira, et al., 2002).

The ability to determine social form and identify Microsporidia pathogens in fire ants has led IFAHIRU scientists to discover that one Microsporidian pathogen of fire ants, *Thelohania solenopsae*, is more prevalent in the polygyne fire ant populations in the United States, while both social forms are infected in Argentina. These results hint at the possible existence of a different *T. solenopsae* biotype infecting monogyne populations, multiple biotypes within *S. invicta*, or that an important vector is involved in transmission to monogyne colonies in South America that is absent in introduced regions. The existence of such variation also presents unique opportunities for enhancing the effectiveness of this pathogen in the United States (Oi, et al., 2004, Valles and Briano, 2004).

Scientists from IFAHIRU also characterized the genetic architecture of a glutathione transferase, which encodes a detoxification enzyme, and a *transferrin* gene in fire ants. These scientists also examined the changes in gene expression of *transferrin* in response to fungal (*Beauveria bassiana*) infection. This study revealed that expression of the gene was up to seven-fold higher in *Beauveria bassiana*-infected ants relative to uninfected ants. Identification and molecular characterization of genes responding to microbe attack or infection, like *Solenopsis invicta transferrin* (SiTf) characterized in this study, provide a more efficient approach to the discovery of potential microbial control organisms for fire ants, as well as new targets for control using novel gene-knockdown approaches (Valles and Pereira, 2005).

The BCPRU extracted the DNA from 3,281 fire ants out of 363 mounds. Polymerase chain reaction (PCR) with microsatellite primers resulted in about 22,000 individual reactions. The products from these have been put through over 7,050 Fragment Analysis reactions to identify gene alleles. Currently, the data resulting from the 2004 field season is being cleaned up, including the running of repeat reactions where there were inconsistencies. Additional samples are being run to fill gaps in the 2003 data set and to sequence questionable alleles. GPS data, collected during the field work phase, has been transformed into a UTM map projection and linked with corresponding specific mound allele data. These studies are a foundation for future research on fire ant movement, variable susceptibility to infection, and other variations that may affect integrated pest management.

3.1.6 Determine the feasibility of genetically transforming screwworm flies into male-only lines.

Summary of Accomplishments:

- Screwworm flies were genetically transformed, providing proof of concept for a new project to develop a male-only strain of screwworm fly.

Impact:
Essential steps were made in the creation of a male-only strain of screwworm fly, an advancement that would save APHIS approximately $4 million per year in production costs.

Screwworm Flies
Transgenic screwworm flies were made using fluorescence producing gene inserted with the ARS-invented piggyBAC transposon system. Five of eight transformed strains had equal fitness to the parental strain, as measured by male competitiveness, pupal weight, and emergence. Such transformation could be useful to the screwworm program in a number of ways, including the use of unambiguously marked flies, genetic sterility, or genetic sexing through female lethal selection (Allen, et al., 2004a, Allen, et al., 2004b, Allen and Scholl 2005). An Interagency Agreement has been finalized between ARS and APHIS to produce a transgenic strain of screwworm fly that produces only males, cutting rearing costs approximately in half. The basic strategy is to produce a strain that has a conditional lethal gene on the female only, the gene being turned on by the absence of tetracycline in the diet. The program is funded for five years, beginning in FY07, with a laboratory male-only strain produced at the end of that time. The strategy uses horn fly as a model so that the work can be done outside of quarantine in Kerrville, Texas. As a result, a male-only strain of horn fly for potential SIT programs will be a spin-off product.

3.2 Neural, Sensory, and Reproductive Biology

3.2.1 Investigate the neural and sensory ultrastructure of ticks and Diptera. In particular, localize and characterize changes in neurosecretion in response to toxic insult.

Summary of Accomplishments:

- Common repellent active ingredients affect vectors in the vapor phase.
- Neurophysiology of ticks was described by studying nerve impulses during feeding and by detection of octopamine receptors in the tick brain.

Impact:
Spatial effects of repellent active ingredients suggest the possibility of new products based on area repellency.

A new class of potential insecticides was discovered.

Ticks, Mosquitoes, and Phlebotomine Sand Flies
Behavioral studies with *Aedes aegypti*, *Anopheles stephensi* mosquitoes and the sand fly *Phlebotomus papatasi* revealed that Deet, SS220, and Picaridin deter feeding as vapors (Klun, et al., 2006). When the compounds were topically applied to the skin of human

volunteers, all three compounds deterred feeding and repelled insects from chemically-treated surfaces without direct contact between the chemical and the arthropod. Complementary behavioral studies of the lone star tick (*Amblyomma americanum*) and the deer tick (*Ixodes scapularis*) with Deet and SS220 (Carroll, et al., 2005). unequivocally demonstrated that ticks respond to and were repelled by the compounds in the vapor phase.

Ticks

The study of tick feeding is of fundamental importance for the understanding of physiological mechanisms that regulate tick-host interaction and disease transmission. Studies of tick feeding on live hosts have revealed several distinct phases of feeding. Blood ingestion in ticks is thought to be accomplished by repetitive suction of blood and salivation. Pharyngeal pump plays a central role in tick feeding. ARS scientists have developed an electrophysiological recording technique that allows simultaneous recording of pharyngeal muscle activity and blood ingestion during feeding of the adult female long star tick, *Amblyomma americanum,* under laboratory conditions for up to 72 hours. Several distinct muscle burst patterns were identified, and a one-to-one correspondence was found between muscle bursts and blood ingestion during particular phases of tick feeding. This technique is a powerful tool for the study of neural mechanisms of tick feeding, as well as the pharmacological action of existing and new bioactive molecules. This could lead to indentification of molecules that have the potential to be developed into new control agents.

Immunocytochemistry was used to identify biogenic amines in the tick synganglion (central nervous system) and other tissues. The goals of this research were: *a)* identification of novel targets for pesticide action in the tick nervous system, and *b)* understanding the mode of action of specific acaricides such as amitraz, and mechanisms of resistance. Serotonin-like and octopamine-like immunoreactivities were successfully detected in the synganglia of the southern cattle tick (*Boophilus microplus*) and two other Ixodid tick species, the lone star tick (*Amblyomma americanum*), and the winter tick (*Dermacentor albipictus*). The identification of serotonin- and octopaminergic neurons in specific regions of the tick synganglia allows elucidation of roles of these neuroactive compounds in regulating tick feeding, mating and other important behaviors. These findings are the first demonstration of biogenic amines in the central nervous system of Ixodid ticks using immunocytochemical techniques. Results from this study provide a basis for further physiological and pharmacological studies of serotonin and octopamine and their receptors in the tick nervous system. This study would ultimately help develop novel acaricides that target specific receptors in the tick nervous system.

3.2.2 Characterize and isolate pheromones and other social cues used by ants and termites in colonization and foraging, and by Diptera and ticks in mating and host finding.

Summary of Accomplishments:

- Pheromones were found affecting ticks, tsetse, mosquitoes, and fire ants.
- Hormonal control of termite social development was demonstrated.

- The relationship between pheromones, neurophysiology, and fire ant behavior was described.
- The term "phagomone" was coined to include all classes of behavior-altering chemicals that affect arthropod biting behavior.

Impact:
New possibilities for chemical manipulation of arthropod behavior were discovered.

The framework for sensory ecology was broadened by improving nomenclature.

Ticks
Work with ticks identified some compounds that either attract or stop movement. *Ixodes scapularis* was attracted to cast tick-larva skins, leading to identification of some purines (guanine, inosine, and xanthine), uric acid, and hematin as specific attractants (Allan, et al., 2002). This discovery was developed to produce an arrestant pheromone consisting of guanine, xanthine, hematin, and 8-azaguanine. Formulated in a proprietary pheromone base ("Last Call") with an acaricide in collaboration with IPM Technologies, Inc., a product was produced that was efficient at killing the ticks (Sonenshine, et al., 2003). Unidentified host substances in dog hair, deer interdigital glands, and chicken feathers were arrestants for *Ixodes scapularis, Amblyomma americanum,* and *Dermacentor variabilis* (Carroll, 2002a, Carroll 2002b). A new technique for evaluation of tick traps was developed, based on the intentional release of *Amblyomma americanum* nymphs (Carroll, et al., 2006).

Termites
The FSTRU showed that juvenile hormone levels increased when a colony of *Coptotermes formosanus* was brought into the laboratory, and that those increased levels induced soldier formation. Work in progress on hormonal control of nymph formation may result in new physiological targets for chemical control.

Mosquitoes
CAIBL initiated work, with funding from the Deployed Warfighter Protection Program, on a wind tunnel system that will track the behavior of a mosquito as it approaches its host. Using a tunnel with laminar flow and artificial blood meal sources, the system has successfully mimicked the host-vector system. The strategy behind this effort is to perform bioassays that measure the many separate behaviors that lead to biting. In this way, we may in the future be able to distinguish chemicals that affect one stage or another of the behavior. A better understanding of biting behavior combined with the means to evaluate it would be a valuable tool. In an effort to stimulate better precision in terminology about biting behavior, the term "phagomone" was coined. Phagomone is intended as a general term for any chemical that influences arthropod biting behavior. Heretofore, the use of the word "repellent" for any chemical that prevents biting has confused the literature with repellent bioassays that are actually measuring a long series of behaviors. It is hoped that adoption of the term phagomone will cause investigators to consider the specific, as well as the general, aspects of the influence of various chemicals.

Tsetse
Components of the cuticular hydrocarbons of the tsetse, *Glossina austeni*, were shown to be sex pheromones produced by the female to induce copulation in the male (Carlson, et al., 2005).

Fire Ants
IFAHIRU defined a new fire ant primer pheromone. Monogyne fire ant colony workers are territorial and aggressive toward members of other fire ant colonies. In contrast polygyne colony workers are not aggressive toward non-nestmates, presumably due to broader exposure to heritable and environmentally-derived nestmate recognition cues (broad template). However, after mating flights, newly mated fire ant queens are heavily predated on by workers from existing monogyne and polygyne fire ant colonies, thus limiting potential reproductive competition. IFAHIRU scientists discovered that existing monogyne and polygyne queens have a remarkable effect on conspecific recognition. After removal of their colony queen, monogyne worker aggression toward non-nestmate conspecifics quickly drops to merely investigative levels; however, heterospecific recognition/aggression remains high. Queenless monogyne or polygyne worker groups were not aggressive toward newly mated queens. Monogyne queenless worker groups that adopted a newly mated queen became aggressive again toward non-nestmate workers and newly mated queens. IFAHIRU scientists proposed that the powerful effect of fire ant queens on conspecific nestmate recognition is caused by a queen-produced recognition primer pheromone that increases the sensitivity of workers to subtle quantitative differences in nestmate recognition cues. This primer pheromone results in the regulation of exogenous reproductive competition in *S. invicta* and when absent allows queenless workers to readily adopt a new queen. The lack of worker-to-worker aggression in polygyne populations falls within the overall conspecific recognition primer pheromone and is a result of the broad template. This extraordinary discovery has broad implication regarding monogyne and polygyne colony and population dynamics (Vander Meer and Alonso, 2002).

The relationship of biogenic amines and fire ant primer pheromones was examined by IFAHIRU and Israeli collaborating scientists. They developed a new microanalytical technique to assay neuro-modulating biogenic amines. This method was used to demonstrate for the first time that the conspecific recognition primer pheromone produced by fire ant queens affects workers by elevating their octopamine levels. This increases their nestmate recognition sensitivity to the subtle intercolony quantitative differences in recognition cues. The effect of the queen's primer pheromone could be duplicated by feeding queenless workers octopamine. These workers maintained levels of aggression comparable to workers with their queen, whereas workers in the absence of their queen were non-aggressive. This is the first discovery of the physiological affect of an ant primer pheromone, and is anticipated to lead to the isolation and identification of the first ant/fire ant primer pheromone. Fire ant primer pheromones have been shown to have powerful regulatory effects on sexual production, dealation inhibition, and other vital population controlling events. Interference of these events could lead to biologically based methods for population reduction and/or reduced reproductive potential of fire ant populations.

Two fire ant dealation (wing removal) mechanisms were found by IFAHIRU scientists. Fire ant queens release a primer pheromone that inhibits dealation of nestmate female alates by presumably suppressing endogenous concentrations of juvenile hormone (JH). Alates cast their wings several days or more after the death or removal of the queen, whereas newly mated alates dealate within one hour after the mating flight. IFAHIRU scientists found that no single pre-mating behavior or combination of behaviors associated with the nuptial flight was found to induce dealation rates comparable with that of newly mated queens. Copulation by itself or in conjunction with other behavioral signals and environmental prompts seems to be critical in causing rapid dealation in newly inseminated alates. In addition, colonies containing alates treated with precosene would not initiate mating flights, nor could they be induced to fly. IFAHIRU scientists suggested that precosene treatment affects the corpora allata (CA), but CA products other than juvenile hormone (JH) or in combination with JH are responsible for rapid dealation after mating. Topical treatment of female alates with JH could not induce dealation rates comparable to that in alates that had mated. Dealation in the two contexts, within the colony and after mating flights, appears to occur via separate mechanisms. Understanding the dealation process could be used to trigger inappropriate dealation and thus decrease the reproductive potential of fire ant colonies and fire ant populations (Burns, et al., 2007).

The first identification of juvenile hormone in fire ants was made. Analysis of hemolymph obtained from sexually mature fire ant female alates from monogyne colonies resulted in identification of juvenile hormone III (JH III). The average amount of JH III was 0.32±0.04 pmol/molof hemolymph. Topical application of 0.038 pmol of JH III was sufficient to stimulate alates to shed their wings in the presence of the queen. The time in which alates were induced to dealate decreased linearly with increasing concentrations of JH III from 0.038 to 3.8 pmol. However, higher JH III concentrations deviated from linearity and did not reach dealation times comparable with those that occur after mating flights. Thus, it appears that the mechanism of dealation that occurs when female alates are out of the influence of their queen is different from the one associated with mating flights. Application of 0.42 mol of precocene II inhibited dealation of alates in queenless colonies. However, this inhibition was reversed after applying 38 pmol JH III to precocene-treated alates. The sizes of corpora allata (CA) from sexuals treated with JH III did not differ from those of controls. However, the sizes of CA were reduced in alates treated with precocene II. This was the first identification of JH in fire ants and further defined two dealation mechanisms (Burns, et al., 2002).

The social organization of fire ants is very complex and depends to a large extent on the breeding system of the ant. Understanding this system is necessary to exploit this vulnerable part of the fire ant's biology. IFAHIRU scientists and a scientist at Eastern Illinois University examined the breeding system of multiple-queen colonies of fire ant by genotyping queens, their stored sperm, and males for a gene thought to code for a pheromone-binding protein affecting complex social behavior. We discovered for the first time that multiple mating of fire ant female sexuals occurs. Our results also led to the proposal that a pattern of differential mortality of two genetically defined sexual types in

colonies may depend on worker genotype frequencies. This selective mortality of male and female sexuals, in turn, is predicted to affect multiple aspects of the breeding system including female-mediated dispersal, mating success, and gene-flow. This information will be useful in planning future, targeted fire ant control research (Fritz, et al., 2006).

The composition of the fire ant alarm pheromone has eluded investigators until recently when IFAHIRU scientists isolated and identified a component of the *S. invicta* alarm pheromone. This compound was shown to elicit an alarm reaction in worker fire ants at the low femtogram levels. In addition, the pheromone was demonstrated to attract worker fire ants using a Y-tube olfactometer. Incorporation of this compound into baits could lead to commercially available fire ant-enhanced bait. IFAHIRU scientists have already demonstrated that the addition of the compound to a standard bait formulation did not affect the foraging ants' uptake or distribution of the bait oil. Interestingly, this same compound was shown to attract fire ant phorid fly parasitoids. Future experiments will determine if phorid fly rearing can be improved by using the alarm pheromone to activate and induce a greater percentage of flies to attack their fire ant hosts.

The BCPRU sequenced the amino acids of the red imported fire ant's pheromone biosynthesis activating neuropeptide.

3.2.3 Develop measurements of electrophysiological activation for use in selecting vector repellents.

Summary of Accomplishments:

- New methods were developed for studying mosquito response to phagomones, with special attention to specific behaviors affected.

Impact:
New methods accomplished more rapid screening of toxicants and repellents.

Mosquitoes
The K& D test module (a clear box divided into six cubes that tests the response of biting arthropods to treatments applied to a human leg in a pattern corresponding to the cubical cages), previously developed and used for quantitative measurement of the efficacy of mosquito repellents on human volunteers, was adapted for *in vitro* evaluation of repellents by coupling the module with a membrane-blood reservoir (Klun, et al., 2005). Performance of Deet, Picaridin and SS-220 insect repellents in the new *in vitro* system was compared with their performance on humans against mosquitoes using our standard *in vivo* system. For each compound, *in vitro* dose-response assays were conducted with compounds applied to cloth positioned over blood reservoirs covered with Baudruche membrane against *Aedes aegypti*. The repellents were also tested *in vitro* against *Anopheles stephensi* and *Ae. aegypti* at a fixed dose of 24 nmol compound/cm^2 cloth over the Baudruche and Edicol collagen membranes. Concurrently, the repellents were tested at the fixed dose using the K & D module on human volunteers. The observed proportions of those mosquitoes species deterred from biting in the dose-response and the fixed doses in the *in vitro* assays showed that assay results were statistically identical to

similar to those obtained using humans, being clearly able to distinguish controls from repellents, and differing only in the ranking of the effectiveness of some of the repellents in all tests against both mosquito species. Dose-response relationships of the *in vitro* and *in vivo* systems were also very similar, although not directly comparable because the data were not collected concurrently. This new *in vitro* assay system can be used in high through-put screening of compounds to identify new repellents having potential for use as topical mosquito repellents on humans.

At an organismal level, work has been done at MFRU comparing the response of different species of mosquitoes to surfaces coated with irritant chemicals. Mosquitoes in a cage are presented with one wall of their cage half-covered with untreated filter paper and the other half of the wall treated with bifenthrin, cyhalothrin, or deltamethrin. The behavior of the mosquitoes was quantified with a video recording, the images from which were analyzed by software that kept track of the landings of each individual. The chemicals varied in irritancy, but most interesting, *Culex quinquefasciatus, Anopheles quadrimaculatus,* and *Aedes aegypti* varied in their responses. For example, the *Culex* tended to rest on the surface for an initial period before starting restless landings and take-offs. The *Anopheles,* on the other hand, was extremely restless from the beginning.

In a second effort that has just begun, single cell recording from individual receptors on a mosquito antenna are being compared following exposure to various host and repellent odors. Working with the Colorado potato beetle as a model, there is some indication that one of the modes of action of Deet is not at the receptor level.

Component 4: Control Technology

The ultimate goal of NP 104 research is to prevent harm by controlling the agent. Although eradication of an arthropod from the United States can be achieved - as was done for screwworm and the one-host tick - suppression is usually more realistic. Chemical control plays an important role, especially during epidemics when danger is great and speed critical. This is particularly crucial in the special role ARS plays in devising means to protect deployed U.S. military. Over the longer period, however, chemical pesticides are often expensive, harmful to beneficial species and the environment, and prone to select for resistance. Precisely targeted methods of delivery, such as toxic baits for termites, or self-sustaining biological agents, such as baculovirus lethal for mosquito larvae, will continue to be major goals. Those entrusted with protecting livestock and people must have a large armamentarium from which to select the most appropriate combination of weapons. It is in the nature of integrated pest management that many different kinds of control techniques are necessary to make an efficient program. Not only can one kind of control be applied when another fails, but some control methods are more appropriate for one situation than another. Although the program stresses non-chemical control methods, the use of rational chemical control is a feature of most successful integrated pest management programs.

Component 4 Goals:

4.1 Chemical Pesticides and Repellents

4.1.1 Develop the capacity to test existing compounds for toxicity against Diptera and ticks that transmit disease. Develop and use computational models for rapidly screening and predicting efficacy of available compounds from configural extrapolation. Devise formulations or combinations of compounds that can be used in regions anywhere in the world, without foreknowledge of resistance patterns.

Summary of Accomplishments:

- Neuropeptide hormones were discovered and sequenced, affecting diuresis in ticks, flies, and mosquitoes.
- The gene responsible for organophosphate resistance was found in ticks, providing a quick and accurate test of resistance.
- Four new chemical classes of potential insecticides were developed, including two that offer promise of variable specificity.

Impact:

The basis of a rapid genetic test for acaricide resistance was discovered.

New classes of insecticides were discovered.

Ticks

The APMRU worked with German scientists to identify the first tick neuropeptide hormone in *Boophilus microplus* (Neupert, et al., 2005). The neuropeptide is part of the chain of regulation of diuresis, a physiologically essential function. The neuropeptide itself is broken down by the tick in order to maintain homeostatic control of water balance, so that the neuropeptide is a poor choice as a toxicant. Currently, five analogues, two of which are not peptides, have been synthesized which mimic the neuropeptide's physiological effect, but which are not subject to the same homeostatic degradation. These analogues were developed with a Dipteran model, but they will probably work on ticks as well.

Although not strictly part of this goal, the study of pesticide resistance is very relevant to the effective use of pesticides. The current program to prevent re-entry of one-host ticks from Mexico to the United States depends heavily on dipping cattle both south and north of the border. *Boophilus microplus* is capable of developing genetic resistance to any of the compounds currently registered for use. KBUSLIRL has studied the occurrence and physiology of acaricide resistance. During the last year, all 12 isolates of ticks that had infested a location in the United States were susceptible to all acaricides. An isolate not associated with tick outbreak was resistant to permethrin, the first such occurrence ever recorded. The laboratory went on to document the distribution of permethrin-resistant ticks in the Mexican state of Coahuila. On a more physiological level, scientists developed a technique to extract and test acetylcholinesterase (the target of organophosphate acaricides) from ticks. Using genetic techniques, KBUSLIRL compared coumaphos-susceptible and resistant ticks, demonstrated overexpression of a gene in organophosphate resistant ticks, cloned the acetylcholinesterase gene responsible for organophosphate resistance in an expression vector, and demonstrated resistance in

the recombinant product. The laboratory sequenced bacterial artificial chromosomes that expressed a pyrethroid-metabolizing enzyme and organophosphate-resistant cytochrome P450. On a hopeful note, the laboratory showed that acaricide resistance lowers tick fecundity. This finding may mean that it would be possible to manage acaricide resistance on a regional scale.

Mosquitoes
The MFRU collaborated with the Department of Chemistry at the University of Florida to use Quantitative Structure Activity Relationship (QSAR) to discover and design effective novel repellents (Katritzky, et al., 2006). The data source was the written record of decades of screening performed at the Gainesville laboratory since the 1950s, mostly in collaboration with the Department of Defense. Computer analysis of tens of thousands of compounds resulted in delivery of 33 piperidines and 12 carboxamides to the MFRU for testing. Through comparative evaluation, these compounds will be used to further refine the QSAR model for repellent discovery.

In a separate effort, analyses of the structural activity of piperidines as toxicants against adult mosquitoes were conducted (Pridgeon, et al., 2007). Five additional chemicals were designed based on these findings and synthesized to confirm the structural activity relationship of the piperidines.

House Flies, Stable Flies, and Horn Flies
The APMRU worked with the KBUSLIRL to discover new neuropeptides in stable flies, house flies, and horn flies (Nachman, et al., 2002, Predel, et al., 2003, Nachman, et al., 2005, Nachman, et al., 2006). This work is significant because it raises the possibility of producing completely new methods for killing flies, as it does for ticks. The APMRU designed and synthesized a modified version of these neuropeptides that selectively inhibit the elimination of fluids and toxins from the house fly; an action expected to allow pesticides to reach higher internal concentrations. Smaller quantities of pesticides would then be necessary to kill a pest fly. Specifically, the analog 'R-LK-CHO' reduced the total amount of urine voided from flies by almost 50%. The activity of R-LK-CHO is selective, acting as a 'magic bullet'. The selective inhibitory activity of R-LK-CHO on housefly tubules represents an important lead in the development of environmentally-friendly insect management agents based on the insect kinins.

4.1.2 Develop and test novel means of applying pesticides and repellents that are more efficient, inexpensive and selective, including the development of toxic baits, methods to bond agents to material, and area repellents.

Summary of Accomplishments:
- New methods were developed for the control of ticks, termites, mosquitoes, biting midges, flies, and fire ants.

Impact:
Significant improvement in control of ticks, termites, mosquitoes, biting midges, flies, and fire ants.

Ticks

The KBUSLIRL worked on a number of new tick control schemes. They developed the "4-poster," a device that attracts deer and then treats them with acaricide. Because deer are a necessary host of the tick vector of Lyme disease, the 4-poster can reduce tick populations to reduce the risk of Lyme disease. Using Amitraz as a toxicant, three trials in Maryland showed a 69-80% reduction in *Ixodes scapularis* nymphs and 95-99% reduction in *Amblyomma americanum* (Carroll, et al., 2002). Feeding by gray squirrels on corn bait dispensed by 4-posters caused accumulations of partially eaten kernels and corn meal in the feeding troughs that could seriously interfere with the operation of the devices. Deer and raccoons, the principal targets of the 4-poster technology, use the devices primarily at night, whereas squirrels feed at the 4-posters during the day. The concept of diurnal corn restriction and a corn restriction mechanism (developed by KBUSLIRL) retrofitted into 4-posters was evaluated by APDL in a field trial in Maryland. Diurnal restriction reduced accumulation of corn in the feeding troughs of the 4-posters, but, importantly, did not reduce the use of the 4-posters by deer and raccoons. Scientists shared the '4-Poster' technology with numerous community and governmental groups, including New York Department of Environmental Conservation, Shelter Island, New York, Tick Committee and community, Fire Island Tick Committee, Ft. A.P. Hill, Adelphi Army Research Laboratories, Goddard Space Flight Center, Aberdeen Proving Grounds, London-Britain Township, Pennsylvania,, C.R. Daniels, Inc. (manufacturers of the '4-Poster'), Y-Tex, Corp. (producers of the 4-Poster 'Tickicide' acaricide). This technology transfer will enable control of ticks that transmit the agent of Lyme disease, human ehrlichiosis, and other tick-borne diseases of humans. The unit also provided technology, training, and strategies for use of '4-Poster' and ivermectin-medicated bait to aid APHIS-VS Cattle Fever Tick Program personnel and Mexican officials in the control of *Boophilus* spp. ticks in south Texas and Mexico. Tick activity and cost models were developed by APDL to aid in making decisions about when to operate 4-posters during winter months when adult *I. scapularis* seek hosts on warmer days (Carroll, 2003, Carroll and Kramer, 2003).

Another device, which is not yet available for purchase, will place a silicon-chip-identified collar on deer attracted to bait. The silicon-chip prevents deer from being collared more than once. The KBUSLIRL bought a commercial device that will enable them to make small lots of pesticide-impregnated collars. This new capability will allow them to try various pesticides and to evaluate collars' effectiveness. Working with the tropical bont tick in the Virgin Islands, KBUSLIRL collaborated with APHIS Veterinary Services to show that injectable doramectrin microspheres kept ticks off cattle for 16 weeks and freed pastures of infestation for at least 12 months. Amitraz collars kept sheep tick-free for 16 weeks. At the request of British nobility, KBUSLIRL developed a method to place acaricide treated wing and leg tags on Scottish red grouse. These emblematic birds are threatened by the louping ill virus transmitted by *Ixodes ricinus*. The technology to control of ticks on Red Grouse and Red Deer was transferred to Lord Charlie Lansdowne, HRH Prince Charles, and other members of the Scottish Game

Conservancy Trust to assist in reducing incidence of louping ill and death of Red Grouse chicks.

Ticks sometimes get carried home on clothing, then bite any member of the household. Cold and hot wash cycles do not kill all nymphs of *Amblyomma americanum* or *Ixodes scapularis*. A better procedure is to use a hot drying cycle, which kills all ticks (Carroll, 2003b).

Termites

In the area of chemical control of termites, Texas A&M University and the FSTRU evaluated bait and treatment systems. The University of Hawaii used ARS funding to examine borate and imidicloprid treatments in Hawaii. A borate treatment prevented infestation for eight years, though it was not able to eliminate an established infestation. It was shown that fipronil- and imidicloprid-treated *Coptotermes formosanus* workers intoxicated an entire colony when they were reintroduced (Shelton and Grace, 2003). Plastic sheet containing 5% lambda-cyhalothrin formed a termite-proof barrier below houses built on cement slabs for more than five years (Su, et al., 2004a).

Biting Midges

Culicoides control was attempted to prevent the spread of epidemic hemorrhagic disease virus (EHDV). Shelters attractive to deer were coated with either permethrin or *Bacillus thuringiensis israelensis* toxin and placed near the larval habitats of the biting midges. This effected some reduction in *Culicoides* in the immediate area.

Mosquitoes

The insect repellents NN-diethyl-3-methylbenzamide (Deet), the racemate and 1S, 2'S stereoisomer of 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220), were tested against *Anopheles albimanus* and *Aedes aegypti* in laboratory human-volunteer assays by CAIBL. Estimated skin doses of Deet or racemic AI3-37220 required to reduce biting by 95% in *Ae. aegypti* were 23 and 35 x nmol/cm^2 skin, respectively, while estimated doses for 95% bite reduction of *An. albimanus* in an approximately 40 year-old laboratory colony established from El Salvador were five times higher at 120 nmol Deet/cm^2 skin and greater than 200 nmol /cm^2 skin for AI3-37220. In tests with the 1S, 2'S stereoisomer of AI3-37220, a newly established colony of *An. albimanus* from Belize bit less aggressively than El Salvador *An. albimanus*. However, the Belize-derived mosquitoes were as resistant as the old El Salvador colony to repellent effects of 1S, 2'S stereoisomer of 2-methylpiperidinyl-3-cyclohexene-1-carboxamide. Earlier workers surmised that usual skin doses of Deet would offer only limited protection against *An. albimanus* in the field. Most recent findings support this speculation, but they also indicate that doses of Deet higher than those needed for protection against *Ae. aegypti* might offer reasonable protection against *An. albimanus* (Klun, et al., 2004).

The effect of mosquitoes on cattle in the United States is not well known. The threat of the introduction of Rift Valley fever virus, which would decimate the cattle industry, increases the need to know how cattle could be protected from mosquito bites. ABADRL performed a large scale trial using ear tags containing PBO and zeta-cypermethrin. They

achieved approximately 80% reduction in bites from *Aedes melanimon* and *Aedes dorsalis* for four weeks, possibly as much from repellency as from toxic action (Lloyd, et al., 2002).

Some of the research in this area was directed at improvement of chemical control (insecticides and repellents). At a most basic level, research was performed to discover entirely new modes of toxic action. One class of potential insecticides developed at the MFRU with funding from the Deployed Warfighter Protection Program, would be initially non-toxic to mammals, easy to modify for different pests, and flexible in its use. A patent application was submitted for this invention. Other research directions included the development of neuropeptide mimics that would disrupt water balance (Area-Wide Pest Management Research Unit, College Station, Texas, with funding from the Deployed Warfighter Protection Program), a study of programmed cell death (MFRU), screening a thousand new compounds for insecticidal and repellent activity (Chemicals Affecting Insect Behavior Laboratory, Beltsville, Maryland, with funding from the Deployed Warfighter Protection Program), discovery of new repellent piperidines (33 compounds), carboxamides (12 compounds), and others (4 compounds) by computer-assisted quantitative structure-activity relationship (MFRU, CAIBL, the Walter Reed Army Institute of Research, and the University of Florida, with funding from the Deployed Warfighter Protection Program), synthesis of five new piperidines toxic to mosquitoes, and assay of new biologically-derived termiticides against mosquitoes (MFRU, with funding from the Deployed Warfighter Protection Program, and the Natural Products Utilization Laboratory, Oxford, Mississippi).

Chemical control depends as much on the manner of application as on the toxicant. One appealing strategy is to create an insecticidal barrier around people or animals that need protection from mosquitoes. MFRU, with funding from the Deployed Warfighter Protection Program, performed trials of the insecticide bifenthrin applied to natural vegetation in Arkansas, as well as developing wax myrtle as a convenient and representative model of southeastern plants that might serve as a substrate for barrier spray. Insecticide treated screens and outer clothing have come into common use for malaria control, for outdoor recreation, and for the military. MFRU developed a quantitative method for evaluating the effectiveness of factory permethrin-treated uniforms for the U.S. Marines and the U.S. Army. Applying this technique, ARS found that quality control is a big issue for factory treatment of military uniforms. The AWPMRU, with funding from the Deployed Warfighter Protection Program, examined the function of pesticide sprayers considered for use by the military, finding that only a few functioned adequately. Building on basic discoveries over the last decade, MFRU developed a functioning dispenser for attraction-inhibitor chemicals that block mosquito recognition of hosts. ABADRL addressed veterinary issues by testing the effectiveness of permethrin spray on cattle. They found that some treatments achieved 90% protection from mosquitoes for six weeks – a valuable piece of information in case of the introduction of Rift Valley fever virus.

Mosquitoes feed on plant-derived sugars and honeydew for nutrition. The toxicity of a range of pesticides combined with sucrose was evaluated for their potential efficacy as

oral baits for three species of mosquitoes. Boric acid with sugar was tested against *Aedes albopictus, Culex nigripalpus,* and *Anopheles quadrimaculatus* (Xue and Barnard, 2003b). Imidacloprid and fipronil were the most effective toxicants for all species with high mortality within one hour of exposure. This provides the basis for development of an attractant/toxic bait for mosquitoes.

Of course, chemicals are not the only tools for controlling mosquitoes. MFRU developed an air-curtain device that keeps mosquitoes out of aircraft. The device could satisfy the regulations of some countries that require disinsection of aircraft before landing. Reluctance by aircraft personnel and passengers to use aerosol insecticides on planes has made the traditional "bug bomb" (also invented by ARS) unpopular. A system to prevent mosquitoes and flies from exiting commercial aircraft at terminals was further refined and is ready for transfer to the commercial sector. Final testing was completed and meetings were held with American Airlines officials to coordinate the protocol for deploying the system in the aircraft immediately after it reaches the terminal. Officials from the Ministry of Health (MOH) of Jamaica and the U.S. Department of Transportation visited the MFRU to tour testing facilities, discuss future plans, and later participated in a demonstration of the system aboard an American Airlines Boeing 757 at Orlando International Airport. If the system is accepted by government officials in Jamaica and other countries currently requiring disinsection of aircraft, the use of pesticides on commercial aircraft would be reduced and perhaps eliminated. A field project to further evaluate this method is being discussed with the Jamaican MOH and American Airlines.

Under the right circumstances, trapping can be used to control mosquito populations. The community of Stephen's Landing on a Florida Gulf Coast island had a huge problem with *Aedes taeniorhynchus*. An experiment with toxic (cyhalothrin) targets baited with carbon dioxide and octenol produced dramatic reductions in mosquito populations during the entire season. Residents were so impressed that they funded a permanent set of traps, even establishing a pipe system to deliver carbon dioxide from a central source. An experimental effort to use commercial mosquito traps on an island near Cedar Key, Florida, reduced *Aedes taeniorhynchus* populations by 80-90% by the end of three years (Kline, 2006).

In response to a request by US Navy entomologists at the Disease Vector Ecology and Control Center (DVECC), Jacksonville, Florida, on behalf of the Equipment Committee of the Armed Forces Pest Management Board, a comparison was made of nine commercial fly traps, including the only model ("Flies-be-gone") that currently has a national stock number (NSN). This trap only collected 7% as many house flies and 16% as many blow flies as the most attractive trap used in this study by MFRU scientists. Another major collaboration with DVECC involved the APMRU Application Technology Research Group using a laser diffraction system to evaluate ULV spray droplet spectra for most types of ULV sprayers used for mosquito control (Hoffman, et al., 2007). Results of this work funded by the DWFP progam showed important contrasts between the ULV spray characteristics of machines likely to be used by the military, as well as for reduction of mosquito biting in situations needing adulticidal control.

House Flies

The MFRU developed a number of new techniques for control of house flies. First, they patented the "Flybrella," a cheap and very efficient method of presenting toxicants to flies so that their dead bodies do not block contact from new flies. Second, they defined the chemicals in molasses that are attractive to flies, providing the means to produce better baits that will not have an offensive odor (Quinn, et al., 2005, Quinn, et al., 2007). Third, they tested a new kind of volatile insecticide that can fumigate spaces such as tents. And, fourth, they evaluated spinosad and imidicloprid as toxicants on a visual target lure, showing that imidicloprid lasts at least one month.

Four candidate insecticides were evaluated for use in visual targets for house flies. In field cages, targets treated with spinosad and imidacloprid in sugar bait provided nearly 100% control after 24 hours. Imidacloprid-treated targets remained effective after one month of natural weathering in the field. Laboratory studies indicate that endosulfan may hold promise for this application without the need for the application of sugar to the targets. Endosulfan, a cyclodiene insecticide with a mode of action similar to fipronil, was recently registered for use in cattle ear tags. Unlike other cyclodienes, endosulfan does not bioaccumulate and may provide an alternative to pyrethroids for contact toxicity. Such a tool may be valuable in resistance management.

Fire Ants

In the area of chemical control, BCPRU developed new antioxidants to extend the shelf life of baits. These antioxidants were derived from fire-ant produced chemicals, an origin that assured that they would not diminish the attractiveness of the baits. The IFAHIRU worked with private industry to develop a new controlled-release formulation for the protection of electrical equipment. Electrical equipment is often infested by fire ants, causing damage and complicating repairs. In collaboration with a CRADA partner, scientists from the IFAHIRU developed a proprietary insecticide/repellent polymer formulation that continues to show effective activity against fire ants in field trials, even after over a year of field exposure. This formulation is specially designed to target the electrical and telephone industries that are plagued by fire ant damage to electrical switching devices and concomitant fire ant control costs. These companies spend hundreds of millions of dollars each year for control of fire ants. This product is scheduled to undergo field trials in cooperation with a major electrical company, summer 2007.

In collaboration with a CRADA partner, scientists from the IFAHIRU developed sustained release formulations of fire ant repellents that were demonstrated to exclude fire ants from areas where they are not wanted for a period of several hours to several months. In further collaboration with the Department of the Army, one of these formulations was used to demonstrate that endangered black-capped vireos at the Ft. Hood military base could be protected from fire ant predation during the bird's breeding season. This is another example of excluding (as opposed to killing) the fire ant from

areas where they are not wanted. BCPRU explored the effect of phthalates as repellents, using a new and sensitive bioassay based on ant digging behavior (Chen, 2005). This finding was an interesting contrast to the lack of repellency of toxic levels of a wide variety of insecticides (Chen, 2006).

Scientists at the IFAHIRU and the University of Florida, Gainesville, Florida, demonstrated that a fire ant bait consistently killed fire ant colonies within three days. Fire ant baits typically required two weeks or more to eliminate colonies. This delay in efficacy is a problem when rapid control is needed in sensitive areas such as playgrounds. How the fast acting bait affected individual fire ants was characterized and may help manufacturers select toxicants to develop faster-acting fire ant bait products (Oi and Oi, 2006). BCPRU found that the season for bait placement can vary greatly within the range of imported fire ants in the United States. In Okahoma, temperature was warm enough for fire ant foraging activity only 25% of the year, compared to 42-59% of the year in Florida (Vogt, et al., 2003).

The BCPRU worked on physical control techniques. They are developing a device to instantaneously heat water for treatment of individual fire ant mounds. They are also working on microwaves as a way to kill ants. In another very practical study, the BCPRU compared the effectiveness and efficiency of broadcast treatments compared to treatment of individual mounds.

4.1.3 Identify and test new classes of topical and area repellents from previously synthesized or natural volatiles. Develop carrier formulations.

Summary of Accomplishments:

- Benzoquinones, callicarpenal, intermedeol, and nepetalactone were among the new repellent active ingredients discovered.
- A new class of phagomones was discovered that inhibits biting by eliminating host recognition.

Impact:

New active ingredients were discovered that might make better repellent products.

The basis of a new form of area repellency was discovered.

Behavior Altering Chemicals that Affect Biting Behavior (Phagomones)

An insight into the natural interaction of vertebrate hosts and arthropods was observed. It was found that benzoquinone defensive substances in millipedes induced a very specific anointing behavior in monkeys. The monkeys would display this behavior when presented with filter paper containing the active components. The benzoquinones repelled ticks and continued to affect individual ticks' behavior for up to three months (Carroll, et al., 2005).

The NPURU, Oxford, Mississippi, originated the idea of testing the extracts of the American beautyberry plant for repellency based on local lore that the leaves would

prevent mosquito bites. Essential oil extracts from *Callicarpa americana* and *Callicarpa japonica* were investigated by the NPUL and CAIBL (Cantrell, et al., 2005). Guided by bioassays using the K&D module with an *in vitro* artificial blood source (Klun, et al., 2005), fractionation of American beautyberry extracts led to the isolation of α-humulene, humulene epoxide II, intermedeol, and a newly isolated terpenoid (callicarpenal). Similar work involving *Callicarpa japonica* resulted in the isolation of an additional compound, spathulenol, as well as the four compounds isolated from *C. americana*. Structure elucidation was performed on all isolated compounds using a combination of GC-MS-EI, high resolution LC-MS-ESI, and one- and two-dimensional NMR experiments. Heretofore, 13, 14, 15, 16-tetranorclerodane, callicarpenal, has never been identified from natural sources. Complete ^{1}H and ^{13}C NMR assignment data are provided for this compound. In repellency studies spathulenol, intermedeol, and callicarpenal showed significant repellent activity against *Aedes aegypti* and *Anopheles stephensi*. APDL in collaboration with CAIBL found that these compounds are also effective against ticks (Carroll, et al., 2007).

Catnip is another botanical source of a potential active ingredient for repellent products. Tests against *Aedes aegypti, Anopheles albimanus,* and *Anopheles quadrimaculatus* showed that nepetalactone was not as potent as Deet as a topical repellent, but that it had greater spatial repellency (Bernier, et al., 2005).

Other new potential active ingredients were developed from a large scale Quantitative Structural Activity Relationship analysis performed at the University of Florida. Analyzing thousands of chemicals that had been screened during 30 years at MFRU, a good predictive structural model was produced based on neural net statistics (Katritzky, et al., 2006).

The laboratory worked with the Walter Reed Army Institute of Research to compare 10% and 20% Picaridin (a recently registered active ingredient), 10% and 20% SS220 (a stereospecific active ingredient discovered by USDA), and the standard military repellent containing 33% Deet (Debboun, et al., 2005, Klun, et al., 2006). The cream formulations were tested every two hours for a period of twelve hours. All were at least 85% effective for twelve hours, with 20% Picaridin and 20% SS220 repelling 100% of the ticks tested throughout the twelve hour period. These formulations showed long-lasting efficacy heretofore unknown for tick repellents intended for use on human skin (Carroll, et al., 2004b, Carroll, et al., 2005b). Work in collaboration with the Uniformed Services University of the Health Sciences showed that SS220 has ten times the spatial repellency of Deet or Picaridin (Grieco, et al., 2006).

The commercial market for repellents is active and dynamic. Although total sales are relatively small compared to other consumer items, public interest tends to be great. A comparison of commercially available topical repellents has been cited often as an unbiased source on duration of repellent products (Barnard and Xue, 2004). ARS was also a major contributor to reviews of literature on repellents' mode of action, efficacy, and techniques (Barnard, 2005, Debboun, et al., 2007).

Phlebotomine sand flies have come into greater prominence in the United States during the current military engagement in Iraq. Thousands of troops have been bitten by these flies, resulting in over a thousand infections with the troublesome parasite, *Leishmania*. MFRU in Gainesville, Florida, tested newly discovered attraction-inhibitor chemicals (chemicals that block reception of host odors to the fly) against *Phlebotomus papatasi* in Egypt with the collaboration of the U.S. Navy.

Prototype devices that release inhibitors at a controlled rate have been developed by BioGuard through a Specific Cooperative Agreement with the MFRU.

Mosquitoes find humans and livestock by using chemicals produced by their potential hosts as a means of orienting them towards the host and obtaining a blood meal. The overall host-seeking process is mediated by a complex interaction of chemicals that attract and hide the host from mosquitoes. The compounds that result in this inhibition of attraction were discovered in the last five years. Recently, 22 additional potent inhibitors were discovered and are being patented. In laboratory studies, these chemicals reduced the host finding ability by preventing 90% or more of the mosquitoes from locating the attractive odors.

4.2 Biological Control

4.2.1 Identify, isolate, cultivate, characterize and test natural pathogens and predators of vectors and pests. Develop methods to enhance the specificity and lethality of control agents.

Summary of Accomplishments:

- A wide variety of pathogens and parasitoids were discovered or developed for biocontrol of termites, mosquitoes, black flies, horn flies, stable flies, house flies, and fire ants.
- *Thelohania* pathogens and *Pseudacteon* parasitoids were applied to the entire southeastern United States for the control of fire ants.

Impact:

Improvements in biocontrol of termites, mosquitoes, black flies, horn flies, stable flies, house flies, and fire ants.

Termites

Biological control of termites was a subject of investigation by FSTRU. Some natural substances were found to be toxic to termites. A commercial cedar extract was of little value for the protection of paper or wood. Working with Nicholls State University, they found that neem tree extract killed obligate protozoal gut symbionts, eventually leading to the death of the host termite. They also found that orange oil extract killed the Formosan subterranean termite in the laboratory. A new species of termite-attacking nematode, *Rhabditis rainae*, was named after the FSTRU scientist who found it. Some very practical results of the biological control research program included the identification of *Melaleuca* and cedar mulches as repellent to termites by Mississippi

State University, the successful treatment of living trees with a foam containing fungal spores of *Paecilomyces*, the identification of toxic components in vetiver grass oil (in collaboration with the CAIBL, Beltsville, Maryland), and the chemical stabilization of a promising toxicant extracted from fungi.

Mosquitoes

A naturally-occurring Mosquito Iridescent Virus (MIV) that kills mosquitoes was isolated by MFRU scientists. This virus is designated as "Regular Mosquito Iridescent Virus" (RMIV) with strains isolated from a number of important mosquito species. In a collaborative effort, the complete genomic sequence of RMIV has been determined with an analysis of its relationship to other iridescent viruses as well as other pathogenic viruses. This DNA virus encodes 126 predicted genes, 27 of which are shared homologues in all currently sequenced iridescent viruses, suggesting a genetic core for the Family Iridoviridae. This fundamental information contributes to our basic understanding of these mosquito pathogenic viruses at the molecular level and will enable a more complete evaluation and understanding how they might be exploited to control of mosquitoes that vector deadly diseases of man and animals.

Scientists at the MFRU have resolved the major structural proteins of a baculovirus that infects and kills mosquitoes (Becnel, et al., 2003, Andreadis, et al., 2003, Perera et al., 2006). Forty-four structural proteins and their genes, some of which play crucial roles in infectivity, host specificity and virulence, have been identified. This work has opened a range of new possibilities for innovative mosquito control. Three proteins crucial for *per os* infectivity (*pif* 1, *pif* 2 and *pif* 3) have been determined which are promising new targets for the development of designer toxicants. 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. The products of this research are an important advancement in developing novel control methods for mosquito vectors.

MFRU made contributions on the systematics of mosquito pathogens in Microsporidia (e.g., *Brachida algerae, Vavraia culicis*, and *Edharzardia aedis*) (Vossbrinck, et al., 2004, Becnel, et al., 2005), the life cycle and biology of the Microsporidian *Amblyospora ferocis* in *Psorophora ferox* (Micieli, et al., 2003), the reclassification of insect pathogens *Helicopsoridium* as algae (Tartar, et al., 2003, Conklin, et al., 2005, Blaske-Lietze, et al., 2006), the biology of a gregarine (Reyes-Villaneuva, et al., 2003), and the discovery of a baculovirus in *Uranotaenia sapphirina* (Shapiro, et al., 2004).

Black Flies

The MFRU discovered a new cypovirus that attacks black flies (Diptera: Simuliidae). This virus was characterized genetically and called SuCPV-20. Biological and molecular studies have determined that this RNA virus has a 10-segmented genome. This new virus from black flies expands the genetic diversity base required to investigate and understand basic mechanisms involved in infectivity and host range that will enhance capabilities for future utilization of these viruses for the control of black flies.

Horn Flies, Stable Flies, and House Flies

Scientists at the KBUSLIRL developed a method for treating cattle that distributed spores of either of two fungi from rubbing bags. They found that spores of Beauveria bassinet successfully controlled the flies and did a better job than spores of Metarhizium anisopliae. Less directly applicable, but potentially important for the future, a collaboration between the AWPMRU in College Station, Texas, German scientists, and the KBUSLIRL, discovered a new neuropeptide in horn flies (Nachman, et al., 2002, Nachman, et al., 2006). The work was funded by a BARD grant at APMRU. This discovery may lead to entirely new and safe insecticides.

The diapriid fly parasitoid *Trichopria nigra* was originally obtained from stable fly pupae in Russia and Kazakhstan. 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 bump fly and flesh flies. Behavioral studies have demonstrated that 24 hours of conditioning of young parasitoids on one species strongly influences the attractiveness of that species in subsequent exposures.

The usefulness of two parasitoids released simultaneously, *Muscidifurax raptor* and *Spalangia cameroni* (Pteromalidae), was demonstrated in a chicken house. The wasps were released after the area was cleaned out. Mortality of sentinel fly puparia was 40% or greater and spot card counts decreased 30% (Summer) or 48% (Fall) (Geden and Hogsette, 2006). A number of parasitoids were evaluated against a range of pest flies. *Muscidifurax raptor, Spalangia cameroni, and Spalangia endius* attacked a wide range of flies (*Musca domestica, Stomoxys calcitrans, Hematobia irritans, Hydrotaea aenescens,* and *Sarcophaga bullata*), but the wasps had lower fecundity on *Sarcophaga bullata. Spalangia nigroaenea* and *S. gemina* attacked *Musca domestica, Stomoxys calcitrans,* and *Hydrotaea aenescens*, but there were fewer on *Hematobia irritans* and almost none on *Sarcophaga bullata. Dirhinus himalayanus* (Chalcidae) was best on *Sarcophaga bullata* and *Hydrotaea aenescens* (Geden, 2006). Matching the right parasitoid with the particular problem fly would apparently be an important part of designing a successful biocontrol program.

One of the challenges of using parasitoids for fly control is that the mass rearing of the parasitoids can become contaminated with unwanted pathogens. MFRU examined the effect of a *Nosema* species on the promising biological control agent, *Tachinaephagus zealandicus* (Encyrtidae). They found that infection decreased both fecundity and longevity (Geden, et al., 2003). They also found that it was possible to cure a *Nosema*-infected colony of *Muscidifurax raptor* (Pteromalidae) by using a combination of heat treatment and rifampicin antibiotic (Boohene, et al., 2003).

Fire Ants

Much effort has gone into biological control of fire ants, which currently is the only possibility of establishing an environmentally sound, self-sustaining solution to the problem. Native ants probably compete with imported fire ants and may prove to be a key factor in preventing re-entry of fire ants into areas that have been effectively treated. Much of the work on native ants was described under risk assessment, but one species,

Pheidole pilifera, was studied in detail to assess its potential effects on fire ants. The IFAHIRU worked with APHIS, Texas A&M University, and Oklahoma State University to spread a natural predator of fire ants and to successfully treat pastures. In both cases, the occurrence of native ants increased.

The IFAHIRU in Gainesville worked in cooperation with the APHIS rearing project and universities and state agencies in 11 states to successfully release two species of South American fire ant decapitating flies (*Pseudacteon tricuspis, Pseudacteon curvatus*) at dozens of locations across the Southeast. BCPRU documented the colonization of 2,249 km^2 in northeastern Mississippi between 2002 and 2004 (Thead, et al., 2005). Phorid decapitating flies are in the genus *Pseudacteon.* They are flies that develop inside the heads of individual fire ants, eventually consuming, decapitating, and killing their prey. The BCPRU continued work with Mississippi State University on the taxonomy, distribution, and ecology of native ants. They completed taxonomic reviews of *Pyramica*, the largest ant genus in Mississippi, and of carpenter ants of the state. In the field, they documented the presence of 166 ant species in Mississippi and discovered a native phorid fly that parasitizes fire ants. The NBCL identified 81 species of bacteria and 200 groups of fungi associated with fie ants, some of which could be important in establishing biocontrol. They also showed that phorid flies imported by the IFAHIRU have become established in southern Tennessee (collaboration with Tennessee State University) and part of Oklahoma (collaboration with Oklahoma State University). *Solenopsis richteri* is common in Mississippi and requires a certain strain of *Pseudacteon curvatus* (the Las Flores, Argentina strain) (Vogt, et al., 2003c). The IFAHIRU worked with SABCL in Buenos Aires, Argentina, Oregon State University, and the University of California Riverside to discover and name a new nematode parasite of fire ants. They successfully developed rearing methods for the nematode.

IFAHIRU also performed studies on the only known fire ant virus, discovered by their unit the previous year. They described the phenology, host specificity, and distribution of the virus, taking advantage of a real-time quantitative PCR detection method of their own invention. They also established that phorid flies are not a vector of the virus. Working on phorids, IFAHIRU got permission to distribute another species (*Pseudacteon obtusus*) of these fire ant parasitoids. They sent material of *Pseudacteon obtusus* to the University of Texas for propagation and distribution. IFAHIRU continued to assist APHIS in their phorid mass rearing operation and also continued to look for more phorid species in South America. Extensive research by scientists in the IFAHIRU has proved that these flies are entirely specific to fire ants and will have no unintended impacts on other kinds of animals or plants, including other ant species. The decapitating flies are rapidly expanding out of their release sites at rates of 10-25 miles per year. They currently occupy more than 100,000 square miles and are estimated to benefit more than 20 million people living in the southeastern United States. A third species of decapitating fly (*Pseudacteon obtusus*) has been approved for field release. IFAHIRU is cooperating with the ARS South American Biological Control Lab (SABCL) in Buenos Aires to collect and evaluate several more species of decapitating flies for release as self-sustaining fire ant biological control agents in the United States. IFAHIRU continued to assist and support APHIS in their decapitating fly mass rearing and release operations.

BCPRU made significant contributions toward improvement of *Pseudacteon* rearing techniques, showing that a dark attack tray improved oviposition rates (Thead and Streett, 2005), that higher humidity improved attack rates (Vogt, et al., 2003a), and that there is hope for storage of pupae through temperature manipulation (Thead and Streett, 2006). *Pseudacteon* culture requires a plentiful supply of fire ants. BCPRU made some practical improvements in these procedures (Vogt, 2003, Chen, 2007).

The high population densities of the red imported fire ant cause medical, agricultural, and other problems, such as shorting out electrical contacts. The electrical problem occurs in a wide variety of equipment, from air conditioning units to stoplight switches. Invading ants are electrocuted and often accumulate in large numbers before repairs are necessary. Scientists at IFAHIRU and Texas Tech University investigated the cause of this phenomenon. Chemical communication systems regulate many activities of fire ant colonies, e.g. recruitment pheromones attract and stimulate workers to follow a trail to food or alternative nesting sites. Alarm pheromones activate and attract workers to intruders or other disturbances. Therefore, electrically stimulated ants may release pheromones that attract additional worker ants into the electrical contacts. Behavioral bioassays and chemical analyses were used to show that electrically-stimulated fire ants release alarm pheromone, venom alkaloids, and detectable amounts of recruitment pheromones. The defensive compounds released in response to electric stimuli can draw more workers toward the source, explaining why fire ants accumulate and foul electrical equipment (Vander Meer, et al., 2002).

IFAHIRU scientists used the discovery that fire ants release a host of exocrine products when electrically stimulated to demonstrate that phorid fly parasites of fire ants are attracted to their hosts when these compounds are released. The electrical stimulation method was adapted to phorid fly mass rearing boxes. Electrical stimulation was subsequently shown to double the number of flies attacking. The number of parasite pupae produced was increased by over 20% when electrical stimulation of workers was used as compared to when it was not used. The recent breakthrough identification of an alarm pheromone component will facilitate the practical use of the alarm pheromone in enhancing phorid fly rearing and in developing phorid fly surveillance and trapping devices.

The IFAHIRU worked with SABCL in Buenos Aires, Argentina, Oregon State University, and the University of California-Riverside to discover and name a new nematode parasite of fire ants. They successfully developed rearing methods for the nematode, which has potential as a self-sustaining biological control agent.

The search for fire ant pathogens that are more devastating to fire ant colonies and offer greater suppression of their populations is important for use in integrated control of this pest. The fire ant Microsporidian pathogen *Vairimorpha invictae* isolated from native ants in South America was successfully transmitted for the first time to uninfected introduced fire ant colonies. The ability to transmit this pathogen in quarantine facilities has allowed host specificity testing to determine if it is safe for field release in the United States as a self-sustaining biological control of fire ants. Transmission studies conducted

by IFAHIRU scientists also documented substantial reduction in growth and survival of infected fire ant colonies (Oi, et al., 2005).

Biological control efforts aimed at managing imported fire ants include the release of parasites and pathogens that may compromise the normal behavior and physiology of fire ant colony brood, workers, queen(s), and sexuals. Field or laboratory-based research in areas unrelated to parasites and pathogens may be confounded and negatively impacted by unknown infection. To compensate for possible diseased colonies it is necessary to know the prevalence of the disease. IFAHI scientists and a scientist at Eastern Illinois University determined that the infection levels of a fire ant Microsporidian pathogen, *Thelohania solenopsae*, were very different in newly mated queens (NMQs) from predominantly monogyne (single queen per mound) and polygyne (many queens per mound) populations, averaging 1.67% and 14.14%, respectively. This information will be useful in future experimental designs because it defines the different probabilities of collecting infected colonies from the two sites and highlights the need to consider potential parasites and pathogens when collecting fire ant colonies for research (Preston, et al., 2007).

Scientists from the IFAHIRU in Gainesville, Florida, in cooperation with ARS scientists at the USHRL in Fort Pierce, Florida, identified for the first time a pathogenic virus (picornavirus) infecting the red imported fire ant. This virus was discovered by detailed examination of a large collection of DNA sequences from the fire ant. Survey data of field-collected colonies supported the existence of a picornavirus in some fire ant colonies in the wild. Importantly, these scientists found that colonies infected with this virus do not reproduce well at all compared to uninfected colonies. Another important advancement by these scientists was the successful transfer of this viral pathogen to uninfected fire ant colonies. More recently, the scientists from the IFAHIRU completed a detailed study describing the phenology, host specificity, and distribution of the virus, taking advantage of a real-time quantitative PCR detection method of their own invention. They also established that phorid parasitoid flies are not a vector of this virus. The discovery of this virus is important because of the potential utility of the virus as a biological insecticide and because techniques developed that led to its discovery and characterization will foster discovery of additional viral pathogens. This virus adds a new biological agent in our arsenal of weapons against imported fire ants (Valles, et al., 2004, Valles and Porter, 2007).

Native ants probably compete with imported fire ants and may prove to be a key factor in preventing re-entry of fire ants into areas that have been effectively treated. Much of the work on native ants was described under risk assessment, but one species, *Pheidole pilifera*, was studied in detail to assess its potential effects on fire ants. The NBCL continued work with Mississippi State University on the taxonomy, distribution, and ecology of native ants. They completed taxonomic reviews of *Pyramica*, the largest ant genus in Mississippi, and of carpenter ants of the state. In the field, they documented the presence of 166 ant species in Mississippi and discovered a native phorid fly that parasitizes fire ants. The NBCL identified 81 species of bacteria and 200 groups of fungi associated with fire ants, some of which could be important in establishing biocontrol.

They also showed that phorid flies imported by the IFAHIRU have become established in southern Tennessee (collaboration with Tennessee State University) and part of Oklahoma (collaboration with Oklahoma State University).

4.2.2 Develop cryopreservation methods for the indefinite archiving of screwworm embryos.

Summary of Accomplishments:

- Screwworm fly embryos were successfully cryopreserved.

Impact:

Cryopreservation may make it unnecessary to develop new strains of screwworms each three years as the current production strain becomes too adapted to factory conditions. Cryopreserved material from the original strain could be revived for fresh material.

Cryopreserved transgenic screwworms will save approximately two years in the development of a male-only strain by providing a transgenic strain for proof of concept.

Screwworm Flies

The techniques for cryopreservation of screwworm fly embryos were developed during the previous five-year research cycle (Leopold, et al., 2001). The work included instructions on reviving embryos and stated that though the survival of cryopreserved embryos was less than the controls, subsequent generations were not negatively affected. Approximately 1,500 embryos representing eight transgenic strains are currently preserved at a facility in Fort Collins, Colorado. These embryos will provide material to develop a colony of transgenic flies in Panama, establishing regulatory permits and proving the concept that transgenic flies can interact vigorously with non-transformed flies in the laboratory and in large-cage trials. The availability of the cryopreserved transgenic flies will accelerate the development of the male-only transgenic strain by providing a transgenic model to be tested prior to full development of the male-only strain.

4.3 Area-wide Control

4.3.1 Integrate biological and chemical suppression techniques with knowledge of behavior and dispersion to develop and test large area prevention and control strategies.

Summary of Accomplishments:

- Area-wide control of the Formosan subterranean termite through the use of non-repellent termiticides in baits effectively reduced the problem even in areas that did not receive treatment.
- Area-wide control of imported fire ants was applied over most of the southeastern United States, establishing new biocontrol agents. Eventually, the ecology of the infested area will be able to provide dynamic control of these pests.

Impact:

Preservation of the French Quarter of New Orleans and many other structures in the city, as well as development of a method for control of the Formosan subterranean termite in any community.

Establishment of biocontrol agents of fire ants throughout the southeastern United States.

Termites

One of the big advantages of the ARS research program on termites is that it includes a major project to control Formosan subterranean termites in the French Quarter of New Orleans, with support to other areas as well. In cooperation with Louisiana State University and NOMTCB, the demonstration project has consistently informed scientists on practical directions for their research, as well as providing a proving ground for application of multiple, integrated methods. Working with the New Orleans Mosquito and Termite Control Board, the program documented significant termite control in the Riverfront and French Market areas (Guillot, et al., 2005), 90% control along the railroad and levee-side planters, and up to 95% control in areas with private properties. Termite activity was completely eliminated in a New Orleans park following three months of treatment with hexaflumuron baits (Messenger, et al., 2005). Outside of New Orleans, FSTRU and TAMU coordinated new guidelines for protection of forests by quarantine and treatment. An areawide program in Florida applied by only 35% of residents resulted in 95% reduction of termite activity in bait stations and 93% reduction in alates (Su, et al., 2004b). Funded by special assistance money, FSTRU successfully developed a termite control program to protect historical buildings in Uzbekistan from the Turkestan termite. The University of Hawaii was able to introduce efficient treatment procedures to operational applicators.

Fire Ants

The population densities of imported fire ants in the United States are five to ten times higher than in South America, most likely due to their escape from natural enemies. Recently, biological control agents became available for introduction into the United States, e.g. phorid fly parasites and a microsporidian pathogen, setting the stage for integrated fire ant management. An areawide fire ant management project proposal submitted by IFAHIRU scientists was funded by USDA/ARS Headquarters to demonstrate control of fire ant populations over large areas using commercially available insecticide bait and self-sustaining biological control agents. Control and treatment demonstration sites (120Ha + periphery) were set up in each of five states (Florida, Oklahoma, Texas, South Carolina, and Mississippi). The control and treatment sites both had bait (hydramethylnon and methoprene) applications, but only the treatment site had biocontrol agents released around the periphery. The area-wide program spearheaded by IFAHIRU scientists has had significant impact. Fire ant population levels have been suppressed below target thresholds in all demonstration sites in pastures. For the first time, fire ant control has been maintained at more than 80% over a total area of about 3,600 acres for four to five years. These properties are now serving as examples for neighboring property owners, and led to continuing expansion of interest in fire ant IPM in different regions of the United States. While the results are still being analyzed, in Florida, fire ant reduction has averaged 88% where the IPM approach was used as

compared to only 71% where fire ants were controlled only by chemical pesticides. In Texas, plots with high phorid fly populations were correlated with lower fire ant populations. Sustainable biological control agents were successfully released into all five states where the area-wide program was implemented, and in dozens of other locations throughout the infested area in cooperation with APHIS and cooperators in each state. Currently, the total area impacted by phorid fly parasites is more than 100,000 mi^2; an area comprising around 20 million people. We anticipate that over the next four to five years the flies will expand their range to over 500,000 mi^2. The area-wide project helped promote inoculations of *Thelohania solenopsae* by university and state Department of Agriculture cooperators in 10 infested states. During the area-wide project it has been documented that the pathogen has become widespread in multiple-queen fire ant populations, where it may be prevalent in well over 60,000 mi^2, with infection rates averaging about 51%. These reductions have translated into tens of millions of dollars saved to the >20,000,000 people in impacted areas (Vander Meer, R.K., et al., 2007). *In* Vreysen, M.J.B., A.S. Robinson, and J. Hendrichs (Eds.), Area-Wide Control of Insect Pests: From Research to Field Implementation, Springer, Dordrecht, The Netherlands (in press). This very complex project won a Technology Transfer Honorable Mention from the Federal Laboratory Consortium for Technology Transfer, Southeast Region. The Project won a Superior in the 2006 Annual ARS Technology Transfer Awards.

4.3.2 Identify and colonize New World screwworm genotypes as potential mass rearing replacements.

Summary of Accomplishments:

- SRU collected and colonized Jamaican strains of screwworm flies, including crosses with established strains. Plans are under way to collect Colombian material for future releases in the barrier zone.

Impact:
Contributed the screwworm strains necessary for maintenance of the barrier in eastern Panama.

4.3.3 Improve the efficiency and reduce the cost of screwworm production.

Summary of Accomplishments:

- Research on the screwworm fly provided practical benefits for the eradication program.

Impact:
Permanent cost savings in the production of sterile male screwworm flies.

Screwworm Flies
The SRU in Pacora, Panama, centers its research on customer needs of those who control this pest by releasing sterile males. The research effort ranges from the highly applied to the basic. During the last year, the SRU worked with Mexican partners (COMEXA) to evaluate a new aerial dispersal system. They also worked with these partners to

determine the level of control achieved in the ongoing eradication effort in Jamaica. That study determined that the population has been reduced by 50%, suggesting that the program requires some adjustment. Following a few years of effort, the production facility at Tuxtla-Gutierrez, Mexico, adapted a cellulose-based diet for the larval flies. Not only does the diet save hundreds of thousands of dollars annually in direct costs, it also eliminates the challenge of disposing of the old diet residue (based on polyacrylamide gel) as a hazardous material. The adult screwworm fly diet was also improved, saving thousands of dollars in materials (Berkebile, et al., 2006).

Appendix 1:
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Appendix 2:
Technology Transfer

The ARS mission emphasizes problem solving for American agriculture. The strategic goals of the agency in the area of animal protection are two fold: to contribute to the body of science and to produce technology of use to agriculture. Contributions to the body of science are documented in peer-reviewed publications. Accomplishments in technology transfer are more varied, reflected in various activities that form part of the path leading from discovery to application. Following is a list of technology transfer achievements as described by each laboratory:

CAIBL:

Patents & Patent Applications: 3

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 7

Scientific Presentations, Public Presentations, Special Events: 23

APDL:

Scientific Presentations, Public Presentations, Special Events: 7

MFRU:

Patents & Patent Applications: 7

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 10

Scientific Presentations, Public Presentations, Special Events: 100

IFAHIRU:

Patents & Patent Applications: 5

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 13

Scientific Presentations, Public Presentations, Special Events: 20

Special Unit Award: All unit Scientists - Headquarters funded: Areawide Control of Fire Ants in Pastures. ($4,902,000). This very complex project won a Technology Transfer Honorable Mention from the Federal Laboratory Consortium for Technology Transfer,

Southeast Region. The Project won a Superior in the 2006 Annual ARS Technology Transfer Award.

Getting the Needed Technical Expertise: ARS has expertise in parasite and pathogen biological control, as well as molecular biology and chemical ecology. ARS scientific expertise was supplemented with: (1) an Agricultural Economist, Texas A&M University, (2) APHIS involvement in large scale phorid fly rearing and in assisting and advising on the use of aerial bait treatments, (3) the education component was directed by a University of Florida Extension specialist which resulted in a website, educational brochures, movies, and other presentation materials. These information tools were used to educate stakeholders, e.g. extension specialists, high value property owners, local government, and the public, about the area-wide IPM program. (4) Each of the Areawide Project's cooperators were charged with the task of developing the within-state infrastructure needed to carry out the complex assessments required for execution of the program and evaluation of program success, (5) Environmental Impact was assessed using ARS and State Cooperator expertise, (6) ARS directed a portion of their research effort to specific problems associated with the Areawide project. All of the above contributed to the successful demonstration of the first continuous areawide integrated management of fire ants in five U.S. states, representing diverse ecological conditions and over a multiple year period.

Technology Transfer Processes: Funding for the project came from ARS. Our state cooperators were funded with ARS money through the development of Specific Cooperative Agreements. ARS developed novel methods for infecting fire ant colonies with the microsporidian pathogen, *Thelohania solenopsae*. These methods were crucial to facilitating the spread of the disease in fire ant populations in the area-wide program. Other researchers have adopted these methods for infecting colonies throughout the range of introduced fire ants. ARS scientists developed a simple and reliable food lure method for estimating fire ant population densities and establishing an action threshold for treatment. Cooperators adopted this method, after ARS demonstrated strong correlations between the new method and the previously used mound count and population index methods. The food lure method reduces the time needed to estimate populations by at least 50%, requires no specialized training, and is easily transferable, simplifying the implementation of fire ant integrated pest management (IPM). The best way to transfer these new technologies/methods was through demonstration and publications, rather than through the invention disclosure/patenting process.

Outcome of Technology Transfer Effort: The area-wide program has had significant impact. Fire ant population levels have been suppressed below target thresholds in all demonstration sites in pastures. For the first time, fire ant control has been maintained at more than 80% over a total area of about 3,600 acres for 4-5 years. These properties are now serving as examples for neighboring property owners, and have provided for a continuing expansion of interest in fire ant IPM

in different regions of the United States. Further examples of impact are listed below.

1) In Florida, fire ant reduction has averaged 88% where the IPM approach was used as compared to only 71% where fire ants were controlled only by chemical pesticides. In Texas, plots with high phorid fly populations were correlated with lower fire ant populations.
2) Sustainable biological control agents were successfully released into all five states where the area-wide program was implemented, and in dozens of other locations throughout the infested area in cooperation with APHIS and cooperators in each state (see Table 1).
3) *Pseudacteon tricuspsis*, the first species of phorid fly released, is currently well established in eight states: Florida, Alabama, Georgia, Mississippi, Louisiana, South Carolina, Texas, and Arkansas (see Table 1).
4) Two biotypes of *Pseudacteon curvatus* have been established in the United States. The first biotype is established on black imported fire ants in Mississippi, Alabama, and Tennessee. A second biotype is established in Florida, South Carolina, Texas, and Oklahoma on red imported fire ants (see Table 1).
5) The total area impacted by phorid fly parasites is more than 100,000 mi^2; an area comprising around 20 million people. We anticipate that over the next 4-5 years the flies will expand their range to over 500,000 mi^2.
6) *Thelohania solenopsae*, a microsporidian pathogen that debilitates fire ant queens and eventually kills the colony is established and spreading in Florida, Texas, South Carolina, and Oklahoma - e.g. 60% increase in Florida's IPM site and natural spread from 0 to 12% infected colonies in the bait toxicant only site.
7) Area-wide project helped promote inoculations of *T. solenopsae* by university and state department of agriculture cooperators in 10 infested states. During the area-wide project it has been documented that the pathogen has become widespread in multiple-queen fire ant populations, where it may be prevalent in well over 60,000 mi^2, with infection rates averaging about 51%.
8) Phorid flies and the T. solenopsae parasite have reduced fire ant populations by at least 1%, and 33%, respectively. These reductions have translated into tens of millions of dollars saved to those in impacted areas.
9) Farm worker safety has been significantly improved due to reduced exposure to fire ants.
10) There have been fewer mechanical and electrical equipment repairs due to fewer fire ants and fewer mounds.
11) Pesticide risks have been reduced by fifty percent.

Table 1. Total area currently occupied by phorid flies and the human population impacted. Five hundred thousand phorid decapitating flies (2 species) were released at the 83 sites in 12 states shown below.

State	Release Sites		Total Area Impacted (square miles)	Human Population in Impacted Area
	P. tricuspis	*P. curvatus*		
Area-wide				
States				
Florida	6	10	35,637	13,420,532
Mississippi	2	2	12,834	1,085,755
Oklahoma	6	3	1,553	48,198
South Carolina	5	1	756	334,609
Texas	19	2	3,404	953,408
Other States (7)	21	7	46,693	4,211,527
Total	59	25	100,869	20,054,000

BCPRU:

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 4

Scientific Presentations, Public Presentations, Special Events: 17

Special Award: An USDA-ARS demonstration project for the suppression of fire ants on pastures was established in five states; Florida, Mississippi, Oklahoma, South Carolina, and Texas in 2002. Biological control agents of fire ants, e.g. microsporidia and phorid flies were used in conjunction with registered bait products to demonstrate area-wide management of fire ant populations on pastures. Currently, phorid flies have been found on approximately 6328.1 square miles, or 4,049,984 acres from the release site. The project received a Superior Effort Technology Transfer Award in 2006.

KBUSLIRL:

Patents & Patent Applications: 1

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 2

Scientific Presentations, Public Presentations, Special Events: 2

Special Award: Technology transfer accomplishments were acknowledged nationally by receipt of an award from the Federal Laboratory Consortium – Midwestern Region for Outstanding Technology. The award recognized research and development of technologies to control of ticks that transmit disease agents to humans.

ABADRL:

Patents & Patent Applications: 1

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 3

Scientific Presentations, Public Presentations, Special Events: 26

SRU:

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 2

Scientific Presentations, Public Presentations, Special Events: 6

Special Impact on Screwworm Eradication Program: Modified the diet for colonized screwworm flies by adjusting the adult diet and the larval medium, resulting in a savings of over one-million dollars per year in rearing costs for the screwworm eradication program.

National Program 104 Technology Transfer Summary for 2002-2006:

Patents & Patent Applications: 17

Significant Agreements (Specific Cooperative Agreements, Cooperative Research and Development Agreements, Interagency Agreements): 41

Scientific Presentations, Public Presentations, Special Events: 201

Appendix 3:
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