VETERINARY, MEDICAL, AND URBAN ENTOMOLOGY (NP 104)

ANNUAL REPORT FOR 2008

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

Background

The goal of National Program 104 (NP 104) is to perform research that contributes to efficient and safe protection of humans and animals from damage caused by arthropods. To achieve this goal, research within NP 104 uses a framework of components in the idealized structure of applied entomology known as Integrated Pest Management (IPM), which provides the basic organization for the program's research. Developed during the 1960s and 1970s, IPM drew on the previous 100 years of accumulated entomological wisdom to produce a methodology that would guide research, education, and application toward solutions that were effective, economical, sustainable, and safe. IPM can be divided into four elements: (1) risk assessment/biology, (2) surveillance, (3) control, and (4) monitoring/sustainability. A complete explanation of IPM can be found in the NP 104 Action Plan for 2009-2013

(http://www.ars.usda.gov/SP2UserFiles/Program/104/NP104ACTIONPLANFY09-FY13Final.pdf). Briefly, *risk assessment* determines whether a problem exists; *surveillance* measures the extent and location of the problem; *control* consists of the many different ways to bring a pest population down to a tolerable level; and *monitoring/sustainability* provides the means to maintain success.

NP 104 Events in 2008

Dr. Ulrich Bernier of the Mosquito and Fly Research Unit, Center for Medical Agricultural and Veterinary Entomology, Gainesville, Florida, won one of six "Superior Effort in Technology Transfer" awards for "...development of bite protection criteria and procedures that the U.S. Marine Corps has implemented to qualify companies that treat Combat Utility Uniforms with repellent." This work applied careful knowledge of mosquito behavior and insecticide chemistry to produce a reasonable, repeatable, and practical evaluation procedure for military uniforms treated with permethrin. It was essential to the U.S. Marine Corps' effort to field pre-treated uniforms in order to provide maximum protection for its troops. The same procedure has been applied to new military uniform textiles, showing that some of them fail to absorb enough permethrin to effectively deter biting arthropods.

The Armed Forces Pest Management Board and NP 104 jointly conducted a workshop on production of innovative solutions for protection of deployed military personnel from arthropod bites that transmit pathogens. This workshop was a review of accomplishments by ARS, university researchers, and industry and was funded by the Deployed Warfighter Protection Program (DWFP). DWFP has been funding this work for 5 years, resulting in more than 100 scientific publications, two new registrations, evaluation of application equipment, and invention of new classes of insecticides. Through an ARS specific cooperative agreement, DWFP completed its first year of

funding IR-4 (the USDA-funded project to register insecticides for specialty crops) to facilitate registration of public health pesticides. Partly in response to issues raised at the DWFP/ARS workshop, the IR-4 Project Management Committee voted at its Strategic Planning Conference to include public health pesticides in its mission.

NP 104 completed a major segment of the national planning cycle during 2008. A retrospective review of the program by an external panel of experts held in August 2007 produced a summary of the strengths and weakness of the program during the previous 5 years

(http://www.ars.usda.gov/research/programs/programs.htm?np_code=104&docid=16494)

. A Stakeholders' Workshop held in October 2007 was attended by approximately 150 individuals representing ARS, academia, industry, producers, and other customers. From this workshop, new goals were developed for 2009-20013, with the Action Plan being finalized in May 2008 following review by ARS scientists and stakeholders. Using the Action Plan as a guide, objectives for each laboratory to be completed during FY 2009-2013 were developed during discussions with individual laboratories and stakeholders. The objectives were sent to the Areas at the end of June 2008 and were used by project teams to write outlines of proposed research. These outlines, completed in October and approved in December, form the basis of the project plans that will be externally reviewed during the summer of 2009 and implemented in September 2009.

We welcome the following new scientists to NP 104:

Jeanne Howell, DVM, Ph.D., Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas

Jerry (Junwei) Zhu, Ph.D., Agroecosystem Management Research Unit, Lincoln, Nebraska

Adalberto Perez de Léon, DVM, Ph.D., Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas (Laboratory Director as of January 5, 2009)

Will Reeves, Ph.D., Arthropod Borne Animal Diseases Research Laboratory, Laramie, Wyoming

And we congratulate

John George, Ph.D., on his retirement from the Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, Texas

Ashok Raina, Ph.D., on his retirement from the Formosan Subterranean Termite Research Unit, New Orleans, Louisiana

Technology Transfer

One of the ways in which ARS scientists provide products that solve agricultural problems is through direct interactions with the public and industry. NP 104 managed seven websites that provided information directly to those who need it to control fire ants and termites. Inventions from the program resulted in six new patent applications, six invention disclosures, and 17 new research agreements with industry.

The inventions most ready for development are advertised to industry as potentially valuable licenses. NP 104 currently lists the following:

Natural Mosquito and Tick Repellent

Promising Biopesticide for Fire Ant Control

Improved Technique for Protecting and Treating Deer Against Ticks

New Method for Controlling Subterranean Termites

New Method for Developing Molecular Pesticides

Environmentally Safe Mosquito Attractant

Technologies to Control Subterranean Termites

New Water-Resistant Ant Bait

Method to Prepare a Natural Mosquito and Tick Repellent from Pine Oil

New Organic Pest Control for Flies and Ticks

Arthropod-Repelling Compounds

Portable Deer Lift

Technology to Help Develop Vaccines for Protecting Cattle Against Scabies

Repellents for Ants

Biological Control for Mosquitoes and Other Insects

Device and Method for Application of Collars to Animals

Repellent for Ants

Notable Accomplishments*

Identified practical problems with control of the cattle fever tick (Components 3, 4). The cattle fever tick is reinvading the United States following more than 60 years of eradication, raising the possibility of reintroduction of bovine babesiosis and making cattle production uneconomical in the southern U.S. In order to contribute toward a solution of this problem, ARS scientists at the Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas, have examined the expressed genes of the nerve concentration known as the synganglion in the cattle fever tick. They found that there are multiple copies of the gene encoding an enzyme essential for nervous transmission and that these copies produce variations of this enzyme. This discovery shows that the tick has the potential of rapidly developing resistance to the principle pesticide used to control it, although field studies by ARS have continued to show that ticks found in infestations in the United States are susceptible to the pesticide coumaphos, an antiacetylcholinesterase toxicant. The scientists have also developed more rapid biochemical tests to detect resistance to this class of pesticides. Treatment failures may be the result of treating cattle during rainy weather, which greatly decreases success. Other work by

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

ARS researchers demonstrated that an alternative ivermectin treatment was less effective than indicated on the label. They also improved the design of the 4-poster for treatment of wild deer (a device that applies a pesticide to deer that are attracted to corn delivered as a bait), preventing feeding on the corn bait by non-target animals. Tests of a combination of pyrethroid and amitraz indicated that the combination might be effective against ticks resistant to either pesticide by itself.

Because the cattle fever tick eradication program is completely dependent on coumaphos, priority research should be applied to finding either alternative chemicals or synergists that will extend the effectiveness of the pesticide. As noted, ivermectin is not performing as well as might be hoped. Although the 4-poster continues to be improved, it is useful only in certain special situations for treatment of deer that support populations of the cattle fever tick. Pyrethroid-amitraz combinations might offer a solution, especially if they were used to take advantage of the physiological selection pressure against amitraz-resistant ticks. The most important impact of these research results is that the pace of research on control of the cattle fever tick needs to be increased significantly in order to avoid disastrous economic consequences for the cattle industry in the southern U.S.

Identified multiple targets for a vaccine against the cattle fever tick (Components 3, 4). Another contribution by ARS scientists at the Knipling-Bushland U.S. Livestock Insects Research Laboratory to the effort to control the cattle fever tick was accomplished in cooperation with the Animal Disease Research Unit, Pullman, Washington. The scientists have used a variety of cutting-edge techniques to find targets in the cattle fever tick that could be attacked by vaccines. These targets are associated with the salivary gland proteins, anticoagulants secreted by ticks during feeding, and tick ovarian proteins. Extensive work toward describing the complicated genome of this tick leverages the biochemical research by identifying the genes that could be used to produce antigens for the vaccine products. An effective anti-tick vaccine could decrease the transmission potential of cattle fever ticks within the United States and synergize efforts to eliminate the ticks by dipping cattle in pesticide solutions. A vaccine applied to wild deer would eliminate deer as a feral source of ticks that later infest cattle.

Improvements in production of sterile male screwworm flies (Components 1, 2, 3, 4). The Screwworm Eradication Program operated by APHIS successfully excludes this pest from all of North America by maintaining a barrier of sterile male flies in eastern Panama. The program is always looking for efficiencies that will reduce costs. ARS entomologists at the Screwworm Research Unit, Pacora, Panama, performed a field study to show that the new production strain of the screwworm fly, Jamaica-06, is just as effective as the strain that has been in use for more than 5 years. The Unit also performed rapid-response research to determine how to reduce ammonia production in the larval medium, a problem that had become a major impediment to operating the new Panama production plant. ARS scientists at the Insect Genetics and Biochemistry Research Unit, Fargo, North Dakota, applied cryopreservation techniques they developed during two decades to practical preservation of screwworm flies. These scientists trained

technicians at the screwworm production plants in Mexico and Panama to perform this procedure reliably.

Jamaica-06 has replaced the old strain, which had become overly adapted to production conditions and less competitive in the field. The selection of a medium that produces a minimum of ammonia has enabled APHIS to plan on scaling up the Panama plant to full production during the first half of 2009. Cryopreservation will eliminate the need to continuously maintain specialty strains of screwworm flies, thus reducing costs. Under development is a mass cryopreservation technique that will also eliminate the need for backup colonies that cost more than \$100,000 per year to maintain.

Multiple approach to biological control of the imported fire ant (Components 3, 4). Since its introduction from South America in the early 1900s, the imported red fire ant has spread throughout the southeastern United States, Texas, and parts of California. The stinging pest now threatens human health, livestock, and wildlife in states further north because of changes in climate, as well as in Hawaii because of frequent shipments from California. ARS scientists at the Imported Fire Ant and Household Insects Research Unit, Gainesville, Florida, and the South American Biological Control Laboratory, Buenos Aires, Argentina, discovered and developed methods for using small flies that attack fire ants. These flies lay an egg on an individual ant, and the fly larva develops inside, eventually killing the ant. These flies have made an impact on fire ant populations throughout the southeastern U.S. During the past year, a fourth species was released, and another species is under evaluation to be certain that it will not affect native species.

Another important natural enemy discovered and developed by these laboratories is a protozoan pathogen of fire ants (*Thelohania solenopsae*). ARS scientists discovered that the parasitic flies become infected with this ant pathogen, helping to distribute the pathogen to other ant colonies. During the past 2 years, ARS scientists discovered two entirely new viruses of fire ants and have now determined the details of the natural infection process and described the protein coat of one of them. Another approach to biological control is the use of substances within the insect to disrupt vital physiological processes. ARS discovered the first neuropeptide in fire ants, a signaling compound involved in pheromone production. One of the reasons that a single species of parasitic fly is not adequate to control the fire ant problem is that each strain of ant is attacked preferentially by a different species, or even strain within a species, of parasitic fly. Comparisons of the genetics of American populations of fire ants with those in South America have shown that 9 to 20 introductions occurred historically, with the state of Formosa, Argentina, as the likely geographical source.

This work improves current successful efforts to reduce populations of imported fire ants throughout the United States by biological control, the only method that could be environmentally and economically feasible. As more strains and species of parasitic flies are established in the United States, the imported fire ants will have increasingly more difficulty outcompeting native ant species, and eventually an equilibrium will be reached in which the imported fire ants are much less abundant. The precise understanding of fire ant genetics enables the targeting of the right strain of parasitic fly or pathogen to the

right strain of imported fire ant. New biological control agents such as neuropeptides and viruses offer the promise of further integration of methods to bring imported fire ants into balance with American ecosystems.

Remote sensing of fire ant mounds (Components 1, 2).

ARS entomologists at the Biological Control of Pests Research Unit, Stoneville, Mississippi, have found that the total volume of fire ant mounds is the same whether the colonies are of the multiple queen or single queen type. The mounds can be detected by measuring five distinct wavebands in the visible, near-infrared, and mid-infrared. Detection and quantification of fire ant mounds from aerial photography will provide an important tool for evaluation of area-wide control efforts, including the successful establishment of biological control agents discovered by ARS. On a smaller scale, detection of mounds within a field or pasture could help target the use of pesticide baits.

Integration of methods to manage Formosan subterranean termite populations in New Orleans (Components 2, 4).

The Formosan subterranean termite became established in the United States in the 1940s. Since then it has proven to be the most damaging termite species where it occurs, threatening the existence of historical buildings in the French Quarter of New Orleans. During 11 years of effort, ARS has managed trial programs to reduce the population of Formosan subterranean termites in the French Quarter to levels that no longer threaten historical buildings. Working with many academic partners and local institutions, ARS researchers have developed methods for risk assessment, surveillance, and control that have finally succeeded in achieving overall population reductions of the termite in the French Quarter. The program monitors flying termites that periodically swarm in a natural process to establish new colonies. These results show a reduction of 44% to 75% of termites. Individual colonies have been targeted with baits that use a minimum of a very safe pesticide. By targeting efforts to locations where colonies are detected by inspection and acoustics, the program has systematically eliminated or controlled colonies from especially problematic buildings and from major sources of termites, such as the Mississippi River levee. Historical buildings in the French Quarter have been saved from destruction, and the strategies developed in the program will be useful throughout the southeastern U.S. where the Formosan subterranean termite occurs.

Discovery of new chemicals for termite control (Component 4).

Scientists at the Natural Products Utilization Research Unit, Oxford, Mississippi, and at the Formosan Subterranean Termite Research Unit, New Orleans, Louisiana, discovered a naturally occurring compound that kills termites, leading to a patent and exploration of analogous compounds. In addition, a fungal extract revealed a single chemical that acted as a powerful attractant and feeding stimulant to the termites. This work may lead to completely new termiticides with low mammalian toxicity. The attractant could be combined with toxicants to make more effective termite control devices.

Tools for the military to prevent fly-borne diarrheal disease of deployed personnel (Components 2, 3, 4).

Filth flies, particularly the house fly, are attracted to the water sources and fecal odors of American military encampments. Moderate and large fly populations increase the risk of diarrheal disease, the primary cause of lost duty time in deployed American military personnel, by amplifying pathogenic bacteria in their guts and by mechanically transmitting the bacteria from feces. ARS scientists at the Mosquito and Fly Research Unit, Gainesville, Florida, systematically compared common types of commercial fly traps, finding a wide variation in efficiency. They showed that the trap currently used by the military is not very efficient. ARS worked with the Armed Forces Pest Management Board to add a more efficient fly trap to the logistics system, which makes the trap available to deployed military personnel. ARS scientists also developed a field expedient fly trap made from a water bottle, demonstrating that simply painting the top of the trap black increased catches by six fold. A new synthetic attractant blend developed by ARS could replace foul-smelling and messy baits currently in use in fly traps. Better and more practical tools for fly control could reduce non-battle disease rates from as high as 20% per week to 4% per week. The availability of healthy military personnel acts as a force multiplier, reducing the number of personnel who must be deployed in order to accomplish the mission.

New chemicals for mosquito control (Component 4).

New active ingredients for mosquito control are seldom developed. As existing active ingredients are eliminated by regulatory concerns and development of resistance, a gap is developing in the ability to control mosquitoes. ARS scientists at the Mosquito and Fly Research Unit, Gainesville, Florida, worked with researchers at the University of Florida to model compounds repellent to mosquitoes. More than 2,000 compounds that had been tested at the Unit formed the database for molecular modeling on a computer. Subsequent synthesis and bioassay of new molecules resulted in seven compounds that are longer-lasting than DEET, the most commonly used repellent active ingredient. Systematic examination of piperidine compounds with modification of some molecular side groups resulted in development of one new molecule that is highly toxic to mosquitoes. Extending efforts with molecular pesticides, scientists showed that the double-stranded RNA that inhibits regulation of programmed cell death selectively kills mosquitoes but not other kinds of flies. A new system for high-throughput screening of trial compounds using first instar mosquito larva was developed and used to screen more than 2,000 compounds. Pesticide development requires years of effort and large investment by industry. By performing the discovery phase of toxicant development, ARS is stimulating industry to develop compounds that are needed to fill in the gaps for mosquito control. New toxicants developed by ARS have been designed to address specific needs of the mosquito control industry, including repellency, specificity, and environmental safety.

Biting midges infected with vesicular stomatitis virus delay feeding (Components 2, 3). Vesicular stomatitis virus appears in outbreaks in the United States at irregular intervals, disrupting movement of animals and causing the need to rule out the symptomatically similar foot-and-mouth disease. ARS entomologists at the Arthropod-Borne Animal Diseases Laboratory, Laramie, Wyoming, demonstrated that a biting midge that transmits vesicular stomatitis virus to livestock did not feed as successfully when it was infected

with the virus. The delay in feeding increases the likelihood that the virus will reach infective levels in more individual midges. Blood feeding is a dangerous time for the individual insect, so a delay increases the likelihood that the midge will be infective by the time it takes a second or subsequent blood meal. Risk estimates of vesicular stomatitis transmission would normally be based on longevity of the midge population taken as a whole. This discovery shows that longevity should be estimated based on the infected population, potentially causing a great change in estimates of risk.

Appendix: Key Publications

The following publications were selected from among all those that appeared in print during FY 2008 on the basis of their impact and relevance. Other important publications are not mentioned here, though they form part of the record of production by NP 104.

Aguilar-Tipacamu, G., Miller, R., Hernandez-Ortiz, R., Vasquez-Pelaez, C., Garcia-Vazquez, Z., Rodriguez-Vivas, R., Fragosa-Sanchez, H., Olvera-Valencia, F., Rosario-Cruz, R. 2008. Inheritance of pyrethroid resistance and a sodium channel gene mutation in the cattle tick Boophilus microplus. Parasitology Research. 103:633-639.

Barnard, D.R., Xue, R., Rotstein, M.J., Becnel, J.J. 2007. Microsporidiosis (Microsporidia: Culicosporidae) affects blood feeding behavior and DEET repellency in *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology. 44(6):1040-1046.

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

Taylor, D.B., Berkebile, D.R. 2008. Sugar feeding in adult stable flies. Environmental Entomology. 37(3):625-629.

Brown, H.E., Paladini, M., Cook, R.A., Kline, D.L., Barnard, D.R., Fish, D. 2008. Effectiveness of mosquito traps in measuring species abundance and composition. Journal Medical Entomology. 45(3):517-521.

Burns, S.N., Vander Meer, R.K., Teal, P.E. 2007. Juvenile hormone III concentrations in female reproductives of *Solenopsis invicta* Buren. Journal of Entomological Science. 42(2):306-309.

Cantrell, C.L., Duke, S.O., Fronczek, F.R., Osbrink, W.L., Mamonov, L.K., Vassilyev, J.I., Wedge, D.E., Dayan, F.E. 2007. Phytotoxic eremophilanes from *Ligularia macrophylla*. Journal of Agricultural and Food Chemistry. 55:10656-10663.

Cantu, A., Ortega-S, J., Mosqueda, J., Garcia-Vazquez, Z., Henke, S.E., George, J.E. 2007. Immunologic and molecular identification of *Babesia bovis* and *Babesia bigemina* in free-ranging white-tailed deer in Northern Mexico. Journal of Wildlife Diseases. 43(3):504-507.

- Carroll, J.F., Benante, J.P., Klun, J.A., White, C., Debboun, M., Pound, J.M., Dheranetra, W. 2008. Twelve-hour duration testing of cream formulations of three repellents against *Amblyomma americanum* (Acari: Ixodidae). Medical and Veterinary Entomology. 22:144-151.
- Chen, J., Cantrell, C.L., Duke, S.O., Allen, M.L. 2008. Repellency of callicarpenal and intermedeol against workers of imported fire ants. Journal of Economic Entomology. 101(2):265-271.
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- Davey, R.B., Miller, R., George, J.E. 2008. Efficacy of amitraz applied as a dip against an amitraz-resistant strain of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) infested on cattle. Veterinary Parasitology. 152(1-2):127-135.
- Defauw, S. L., Vogt, J. T., Boykin, D. L. 2008. Influence of mound construction by red and hybrid imported fire ants on soil chemical properties and turfgrass in a sod production agroecosystem. Insectes Sociaux. v. 55, no. 3.
- Doolittle, M., Raina, A.K., Lax, A.R., Boopathy, R. 2008. Presence of nitrogen fixing *Klebsiella pneumoniae* in the gut of the Formosan subterranean termite (*Coptotermes formosanus*). Bioresource Technology. 99:3297-3300.
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- Gilbert, L.E., Barr, C.L., Calixto, A.A., Cook, J.L., Drees, B.M., Lebrun, E.G., Patrock, R.J., Plowes, R.M., Porter, S.D., Puckett, R.T. 2008. Introducing Phorid fly parasitoids of red imported fire ant workers from South America to Texas: outcomes vary by region and by Pseudacteon species released. Southwestern Entomologist. 33(1):15-29.
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- Kline, D.L., Allan, S.A., Bernier, U.R., Welch, C.H. 2007. Evaluation of the enantiomers of 1-octen-3-ol and 1-octyn-3-ol as attractants for mosquitoes associated with a freshwater swamp in Florida, USA. Medical and Veterinary Entomology. 21(4)323.
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