U.S. Department of Agriculture
Agricultural Research Service

Retrospective Review
National Program 104

Veterinary, Medical, and Urban Entomology

Vision: Enhancing the health and well-being of humans and other animals through the reduction of arthropod impact.

Agenda

Welcome and Charge

- Jeff Silverstein, Deputy Administrator, Animal Production and Protection
- Bob MacDonald, Coordinator, Partnerships and Grants, Office of National Programs

Introductions

- Uli Bernier, National Program Leader, Veterinary, Medical, and Urban Entomology
- Major Dhillon, Northwest Mosquito and Vector Control District, Panel Chair

Program Information and Summary

- Uli Bernier

Accomplishment Stories: Components 1,2,3

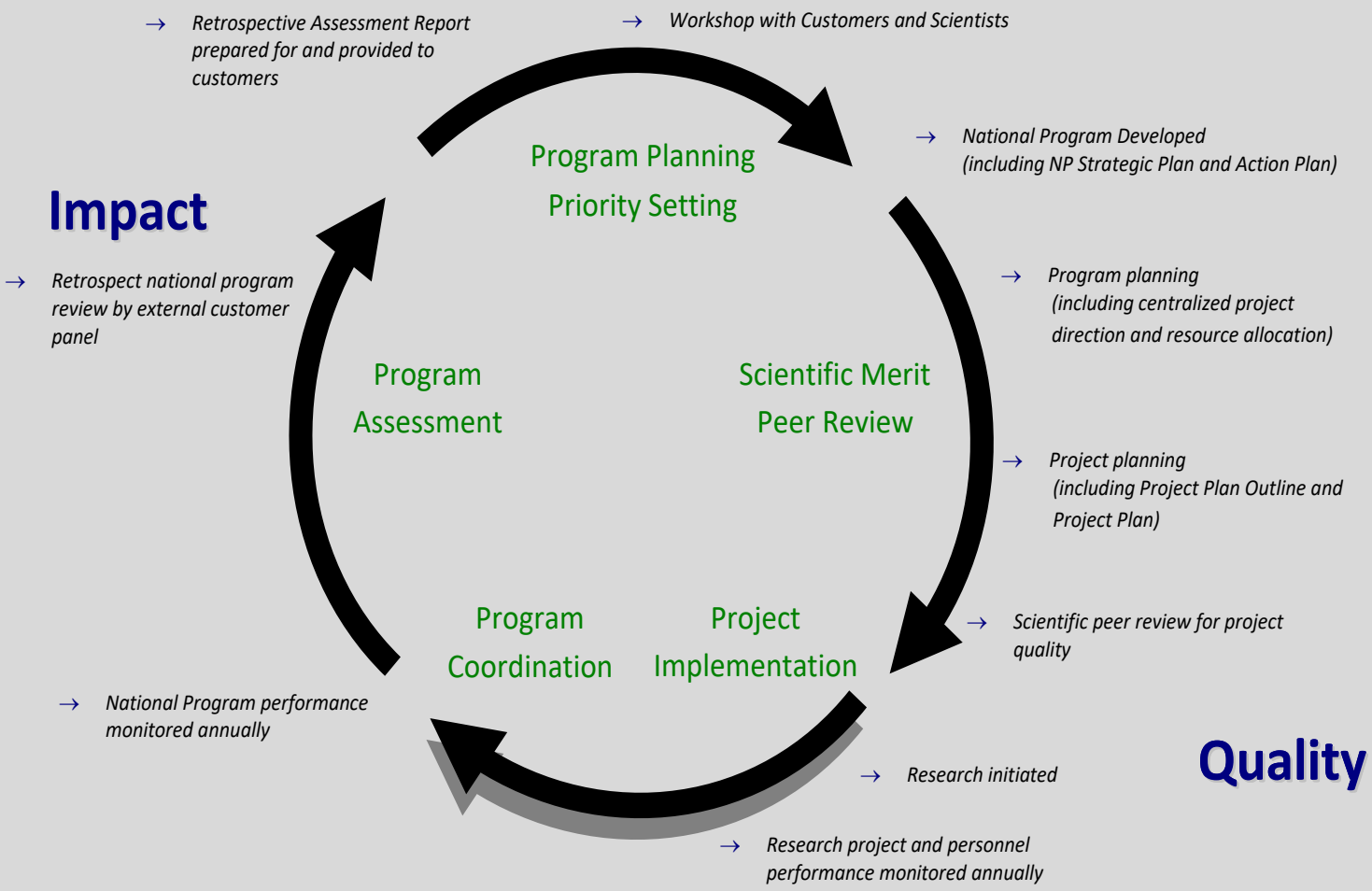
- Uli Bernier

Panel Discussion (Panel Only)

Panel Report and Debrief (Panel, ONP)

ARS National Program Cycle

OMB Research & Development Investment Criteria Relevance



Retrospective Analysis

Focus on 5 years preceding completion of 3rd year cycle;

- 2012-2017
- Final 2 years of previous cycle, first 3 years of current cycle

Conducting an in-house program assessment and documenting research accomplishments and/or progress on the National Program Action Plan for presentation to external reviewers;

Conducting an external review of accomplishments and/or progress, based on the preceding documentation, focused on the research's relevance, quality, and impact;

Recording the results of the review; and

Informing ARS leadership of evaluation results.

Interactive Approach

A PowerPoint document is prepared for presentation to the Evaluation Panel

- Summary Information
- Program Linkages
- Accomplishment Examples

Permits direct interactions between panel members and ARS Scientists, National Program Leaders, providing an opportunity for improved communication

The presentation is supplemented by other documentation provided prior to the Panel Meeting

Online > Documents > Presentation

Effectively reduce reviewer time in reviewing and travel

Program Linkages

ARS Strategic Goal Area 4: Animal Production & Protection

Goal 4.2: Prevent and Control Pests and Animal Diseases that Pose a Threat to Agriculture, Public Health, and the Well-Being of American Citizens.

Goal 4.2: Prevent and Control Pests and Animal Diseases that Pose a Threat to Agriculture, Public Health, and the Well-Being of American Citizens.

The goal of the ARS animal protection research programs is to protect and ensure the safety of the Nation's agriculture and food supply through improved disease detection, prevention, control, and treatment. Basic and applied research approaches will be applied to solve animal health problems of high national priority. Emphasis will be given to methods and procedures to control animal diseases through the discovery and development of:

- Diagnostics and tools for identification of pests/vectors
- Pesticides, repellents, attractants, traps, and other innovative products for pest/vector control
- Animal, pest, and vector genomics applications
- Disease management systems and integrated pest/vector management systems
- Applications of global information systems
- Chemical ecology of pests and vectors

Goal 4.2: Prevent and Control Pests and Animal Diseases that Pose a Threat to Agriculture, Public Health, and the Well-Being of American Citizens.

Animal protection national programs have eight strategic objectives:

1. Develop an integrated animal, arthropod, and microbial genomics research program
2. Launch research programs to provide alternatives to antibiotics in food animal production
3. Build a technology-driven vaccine and diagnostic discovery research program
4. Develop core competencies in field epidemiology and predictive biology
5. Develop expert collaborative research laboratories recognized by the World Organization for Animal Health (OIE) and the United Nation's Food and Agriculture Organization (FAO)
6. Develop a model technology transfer program to achieve the full impact of our research discoveries
7. Perform the full spectrum of research for improvement of veterinary, public, and military entomology
8. Develop safe and effective methods for prevention of damage caused by arthropods to homes and households

2012 – 2017 USDA ARS Strategic Plan Area 4

Goal 4.2: Prevent and Control Pests and Animal Diseases that Pose a Threat to Agriculture, Public Health, and the Well-Being of American Citizens.

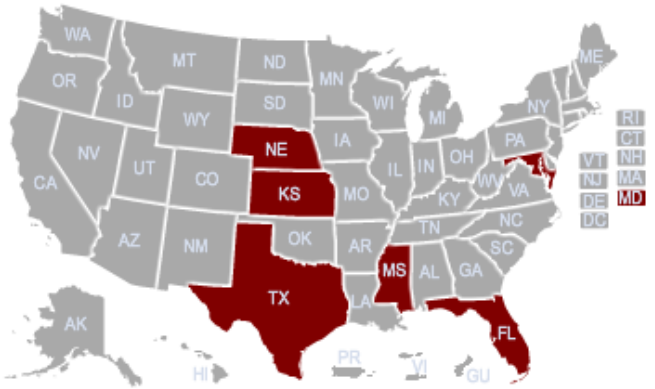
Performance Measure 4.4.2: Provide scientific information to protect animals, humans, and property from the negative effects of pests and infectious diseases. Develop and transfer tools to the agricultural community, commercial partners, and government agencies to control or eradicate domestic and exotic diseases and pests that affect animal and human health.

Program Information

Personnel and Locations

Fiscal Year	Number Scientists	Number Locations	Number Research Projects
2012	55	8	13
2013	46	8	11
2014	40	7	13
2015	40	7	13
2016	41	7	13
2017	40	7	11

Projects in this Program - by State



Legend:

Maroon = click to see related projects in these states.

Gray = No related research in this state.

Program Information

External Funding Received

Sources of External Funding to NP 104 Scientists 2012-2017						
Industry	International Organizations	Federal Government			University	State
		USDA APHIS	USDA NIFA	Other Federal		
47	6	8	1	47	15	1

Program Information

Technology Transfer, Collaborations, and Publications

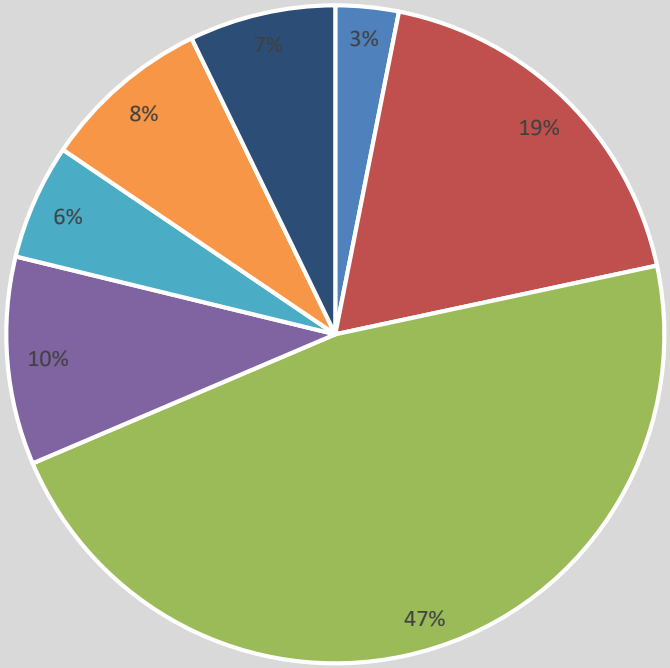
Fiscal Year	Applications for New Patents	New CRADAs	MTRAs MTAs	Refereed Journal Articles	Articles per SY
2012	3	6	26	124	2.25
2013	6	6	49	94	2.04
2014	2	3	33	83	2.08
2015	3	1	13	95	2.38
2016	7	4	23	79	1.93
2017	6	1	17	87	2.18

Mentoring and Editorship

	Postdoctoral Fellows	Graduate Students	Undergraduate Students	Visiting Scientists	Editorships
Total 2012-2017	29	75	196	47	41

International Collaborations

In National Program 104, for the years 2012 to 2017, there were 256 research collaborations with other nations. This large number of collaborations documents the strong international impact of NP 104 research. The data show that the majority of the collaborations were with countries in Europe and Central and South America followed by Asia, Oceania, North America and Africa.



International Collaborations

Europe	Central & South America	Asia	Africa	Oceania	North America
DENMARK	ARGENTINA	BAHRAIN ISLAND	EGYPT	AUSTRALIA	CANADA
FRANCE	BOLIVIA	CHINA	KENYA	FRENCH POLYNESIA	CUBA
GERMANY	BRAZIL	INDIA	MOROCCO	NEW CALEDONIA	MEXICO
GREECE	COLUMBIA	ISRAEL	SOUTH AFRICA	NEW ZEALAND	
ITALY	COSTA RICA	JAPAN	TUNISIA		
SPAIN	ECUADOR	OMAN			
SWEDEN	PANAMA	PAKISTAN			
SWITZERLAND	PARAGUAY	PHILIPPINES			
UKRAIN	PERU	SAUDI ARABIA			
UNITED KINGDOM	URUGUAY	TAIWAN			
		THAILAND			
		UNITED ARAB EMIRATES			

NP104 Action Plan
Research Components

Component 1: Medical entomology for the public and the military

Component 2: Veterinary entomology

Component 3: Fire ants and other invasive pests

Component 1: Medical entomology for the public and the military

Problem Statement 1A: Mosquitoes

Problem Statement 1B: Flies

Problem Statement 1C: Sand flies

Problem Statement 1D: Ticks

Problem Statement 1E: Bed bugs

Novel Toxicants for Efficacy Against Insecticide Resistant Vectors (1A)

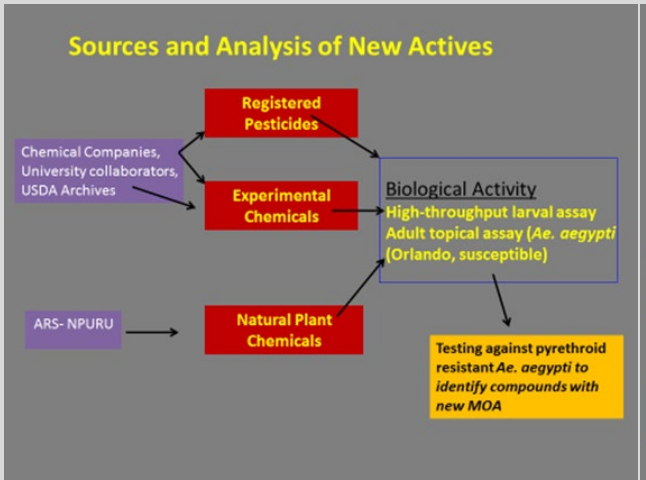
Statement of Problem: Effective mosquito control requires novel toxicants that are effective against pesticide resistant populations. Efforts are focused on discovery of novel adult and larval toxicants as well as characterization of the resistance patterns in the vector.

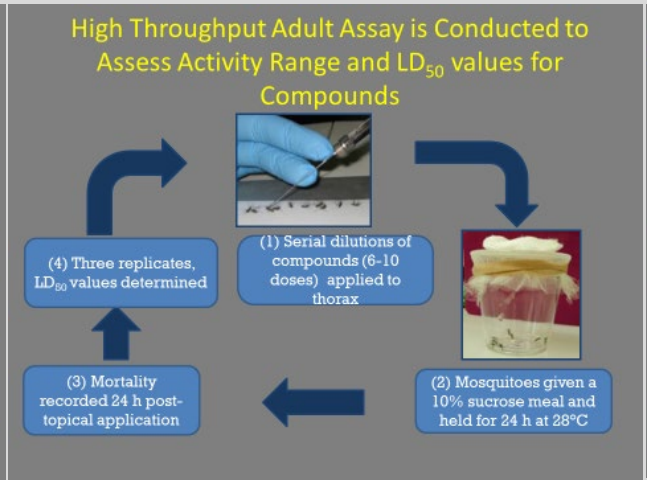
Accomplishments:

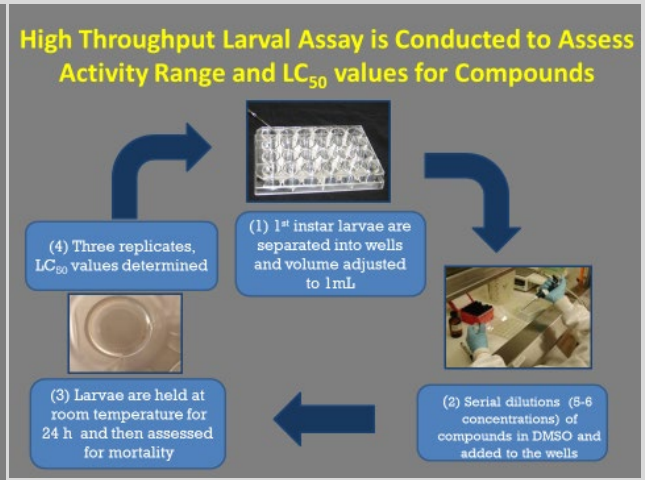
- 1319 samples screened from 12 collaborating institutions.
- Pyrethroid resistance characterization for 18 *Aedes aegypti* strains and 9 *Ae. albopictus* strains
- Genetic resistance characterization for 62 strains of *Ae. aegypti* from FL, 8 from TX, and 7 from AZ.
- Puerto Rico resistant strain founded, characterized and submitted (BEIResources #NR-48830).
- Production and provision of over 750,000 PR pyrethroid-resistant strain eggs to industry purchasers.
- Cone bioassay testing on indoor residual spray panels in collaboration with NECE/CDC.

Impact:

- 18 publications on toxicology with identification of several new lead compounds
- 3 publications on insecticide resistance
- Resistance profiles provided to mosquito control districts and state agencies in Florida, Texas and Arizona to help direct effective mosquito control strategies.





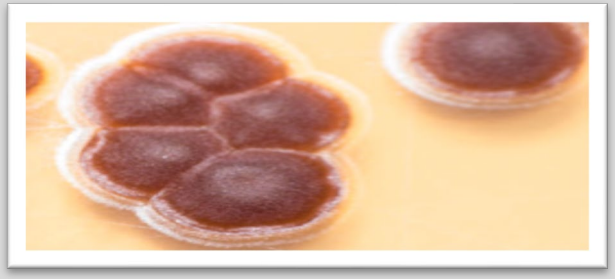


Microbial Bioinsecticide Development from *Streptomyces* sp. CMAA1322 (1A)

Statement of Problem: Highly effective and safe mosquito larvicides are needed to help manage *Aedes aegypti* within the US. This mosquito transmits dengue, chikungunya, zika and yellow fever.

Accomplishment: A new *Streptomyces* sp. was isolated from a soil sample from a tropical dry forest (Caatinga) in the Brazilian semiarid region. Preliminary research resulted in the isolation of natural compounds with LD_{50} values of <1 ppm against *Ae. aegypti* larvae.

Impact: Microbial bioinsecticides represent environmentally friendly alternatives to conventional insecticides. The genome has been sequenced for this new species. Development of a microbial bioinsecticide is in the early phases. Optimization of growth conditions and metabolite production is underway.



Microbial Bioinsecticide

Gene Silencing Technology For Mosquito Control (1A)

Statement of Problem:

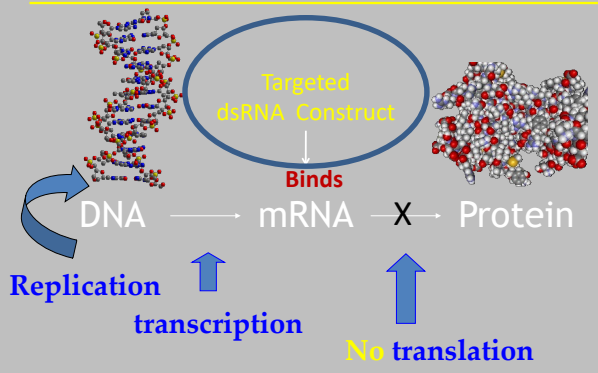
- Limited number of public health pesticides are available for controlling medically important vectors.
- Novel approach is based on the technology that allows for the specific silencing of genes critical to survival of the target vector species.
- Double stranded RNA (dsRNA) and the process of RNA interference (RNAi) is used to selectively silence (reduce) critical gene products (proteins) to debilitate the mosquito vector and prevent disease transmission.

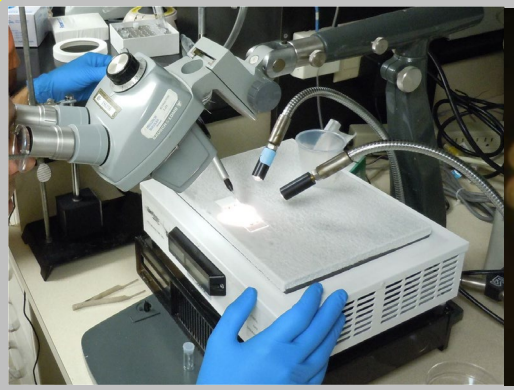
Accomplishment:

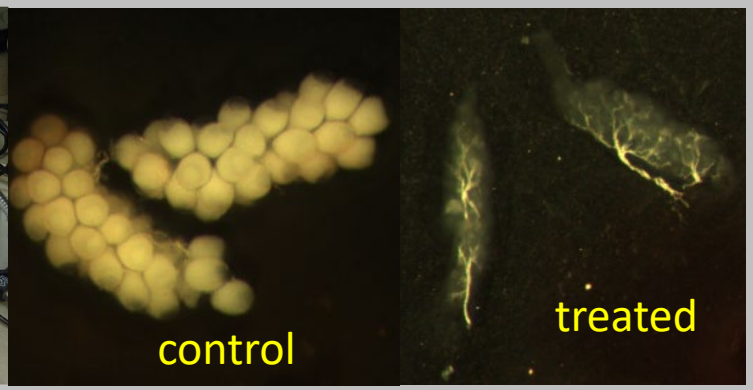
- Targeted major cellular systems including the proteasome, the ribosome, ESCRT system, clathrin transport, transcription machinery, cytoskeleton, and apoptosis.
- More than 70 dsRNA and siRNA designed and tested for activity.
- 3 triggers that cause a loss of fecundity in *Aedes aegypti*.
- 2 triggers that cause a loss of fecundity in *Musca domestica*.

Impact:

- Target selection, dsRNA format, dose, and tissue susceptibility are critical parameters that must be considered for development of effective RNA based pesticides to control mosquito vectors.
- 4 publications related to dsRNA, 1 submitted dsRNA review.
- 2 patent applications; one accepted patent, one new application drafted.







Project SeaRaven: Collaborative Project with NECE (1A)

Statement of Problem:

- Military and civilian personnel face many insect-vectored diseases in field deployments and overseas postings including dengue, malaria, cholera, leishmaniasis.
- Insect vectored diseases reduce readiness, fitness, and mission success while increasing costs and personnel losses.
- Currently, no system exists to rapidly determine which vector species are present and whether they are infected with harmful pathogens. This problem is also faced by civilians and agriculture, where vectored diseases and food-borne pathogens are concerns.

Project goals:

- Use third generation nanopore sequencing technology to rapidly identify species of dipterans, acarines, and pathogens present in standard insect surveillance samples
- Identify hosts and pathogens in surveillance samples
- Identify the presence of resistance genes in hosts or pathogens.
- Develop a standalone database and bioinformatics pipeline to perform real-time analysis of the sequencing data.
- Provide the sequencing system and analysis pipeline in a mobile format that can be rapidly deployed.
- Validate the system under field conditions with military units.

Accomplishment:

- Produced and performed proof of concept on mobile system
- Deployed prototype system with Navy entomologist for a 4 month mission in the Caribbean
- Preliminary data analysis pipeline written

Impact:

- Rapid analysis of species present in a collection and the status of pathogens and microbiomes is important in agriculture, biosecurity, and food safety
- Technology used in this project is equally as applicable to these civilian fields as it is to military use.

Suitcase-sized field deployable prototype sequencer setup

Volatiles from Flowering Butterfly Bushes that Affect Mosquito Oviposition Behavior (1A)

Statement of Problem:

- Effective control of invasive species requires effectual detection technologies.
- The problem is that invasive species like *Aedes albopictus* utilize cryptic habitats for oviposition.
- *Aedes albopictus* utilizes skip oviposition behavior, *i.e.*, deposits eggs in many different containers.

Accomplishments:

- Volatiles from flowering *Buddleja davidii* were found to impact the skip oviposition behavior of the Asian Tiger mosquito, *Aedes albopictus*. Normally this mosquito will deposit her eggs in multiple containers. In the presence of flowering butterfly bushes or their volatiles she will deposit most of her eggs in a single container. This will allow the development of innovative surveillance and/or population management strategies.

Impact:

- 3 published manuscripts and 4 presentations at national and international scientific meetings.
- 1 more manuscript in preparation on impact of these flowering plants on attracting predators such as *Toxorhynchites,* which produce predatory larvae that feed on the larvae of the invasive species.
- These data have been presented to operational mosquito workers in various workshops.

Buddleja davidii flowering

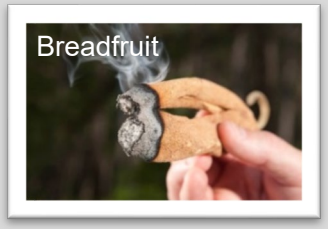
Buddleja davidii flowering attracting *Toxorhynchites* male

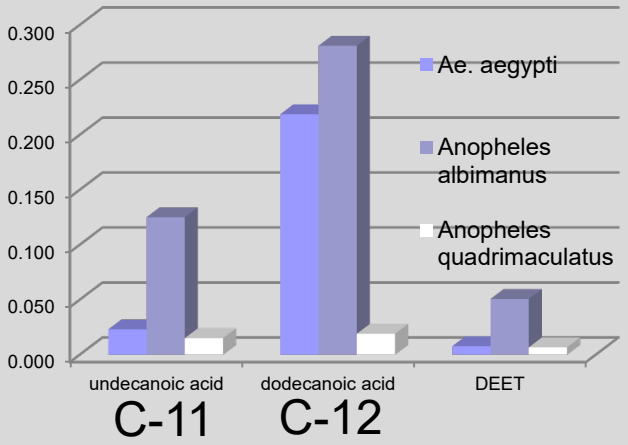
Fatty Acid Based Mosquito Repellent Derived from Traditionally Burned Plants (1A)

Statement of Problem: The use of synthetic insecticides to control pests has met with undesirable toxicological and environmental effects and evolved resistance in pest populations. Natural products may provide a significant source of novel candidates that may be developed as intact biochemical biopesticides.

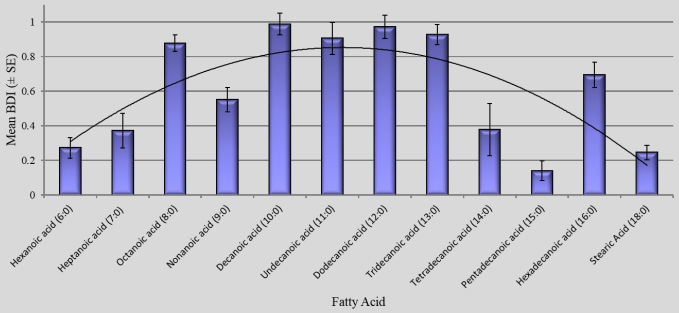
Accomplishment: Folk remedy plants *Jatropha* sp. and Breadfruit led to the discovery natural mosquito repelling triglycerides and the validation of the spatial repellent nature of these traditional remedies with fatty acids as the active components. Through secondary studies this also led to an optimum carbon chain length for enhanced mosquito repellency for bioactive fatty acids.

Impact: Results from these studies continue to indicate the high repellent properties of C11:0 and C12:0 as compared to commercial active ingredients DEET and picaridin. This research has led to the development of prototype mosquito spatial repelling devices currently being developed in-house and by commercial partners.





Cloth Patch-Mosquito Minimum Effective Dose (mg/cm²)



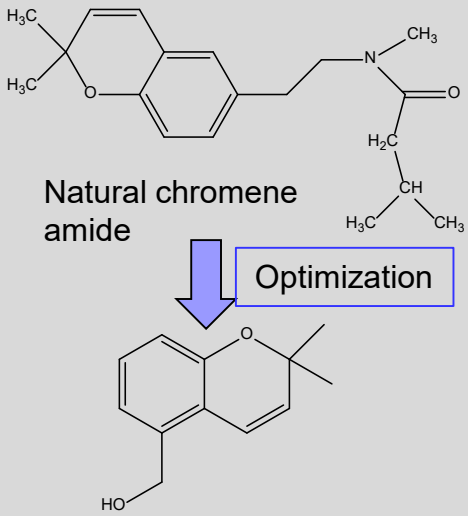
Mean BDI (± SE) values of saturated fatty acids at 25 nmol/cm^2 vs *A. aegypti*

Synthetically Optimized Mosquito Repellent based on a Natural Chromene Analogue (1A)

Statement of Problem: Mosquitoes are primary vectors that transmit pathogens that cause many diseases such as malaria, yellow fever, Zika, and dengue fever. Some mosquitoes have developed resistance to currently available insecticides, and thus better products are needed to prevent bites from these insects to reduce the risk of disease transmission to humans and other animals.

Accomplishment: Developed novel mosquito repelling compounds that are highly effective against *Aedes aegypti* and *Anopheles albimanus* mosquitoes. Potent compounds were synthesized with longer duration of protection from mosquitoes than the commercial product DEET. These chromene compounds are more effective repellents and can be used by the general public topically and by the military for face paint, uniforms and bed net treatments.

Impact: Patent has been awarded for this technology and an application for a license has been applied for by a potential partner.



SYN131-1, **6 days of protection**, MED=0.009 mg/cm2


US009763444B2

(12) United States Patent
Meepagala et al.

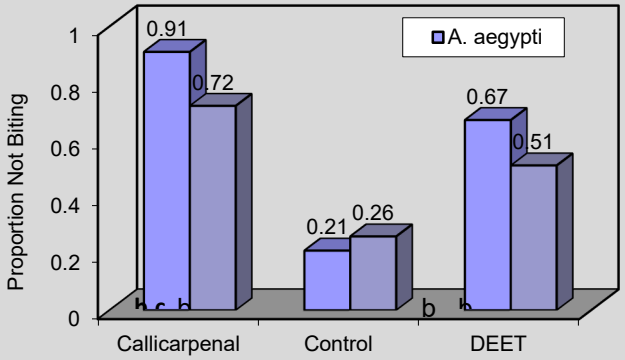
(10) Patent No.: US 9,763,444 B2
(45) Date of Patent: Sep. 19, 2017

Arthropod Repellents from the American Beautyberry (1A,1D)

Statement of Problem: The use of synthetic insecticides to control pests has met with undesirable toxicological and environmental effects and evolved resistance in pest populations. Natural products may provide a significant source of novel candidates that may be developed as intact biochemical biopesticides. Consumer demand for natural product-based topical repellents is increasing.

Accomplishment: Two of the isolated repellent compounds callicarpenal and intermedeol have been shown to be as active as DEET in human bioassays in a dose-response manner against the deer tick. Callicarpenal was shown to be as effective as DEET against *Ae. aegypti* and *An. stephensi*

Impact: Callicarpenal, was the subject of a patent application due to its excellent repellency towards mosquitoes and ticks. Commercial partners completed total synthesis and in-house evaluations for use as a novel natural active repellent ingredient.



Callicarpenal Against Mosquitoes in K & D Module Assay

	15 m.	3 h.	4 h.
Callicarpenal	98.0	100	53.3

Callicarpenal Against Black-Legged Tick Duration Assay at 155 nmole/cm2 Percent Control

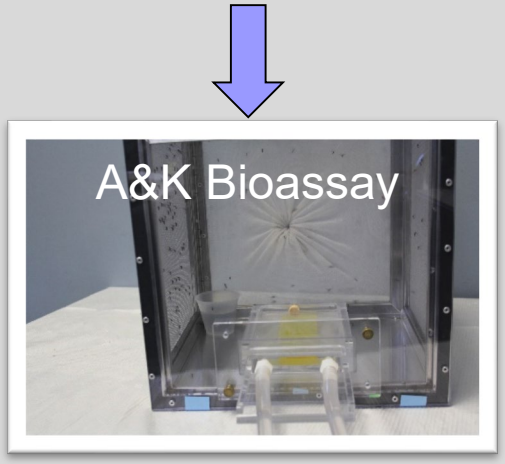
Large Cage *in-vitro* A&K Bioassay for Topical Repellent Discovery (1A)

Statement of Problem: Mosquitoes vector many pathogens that cause human diseases. Repellents play a significant role in reducing the risk of these diseases by preventing mosquito bites. Bioassays for new repellents that require minimal amounts of sample and no human subjects are needed to streamline discovery efforts.

Accomplishment: This particular bioassay fills a gap for the evaluation of the effectiveness of mosquito repellents and is intended to complement both the K & D module bioassay and the *in vivo* cloth patch bioassay. The bioassay addresses some of the limitations of existing bioassays and will serve as a tool to produce data that can directly be translated into the *in vivo* cloth patch bioassay.

Impact: There is no need to involve human subjects, who are not easily available and are one of the biggest resources in *in vivo* bioassays. US EPA has significant interest in a non-human subject assay to determine repellent efficacy.

K&D Module Bioassay



Cloth Patch Bioassay

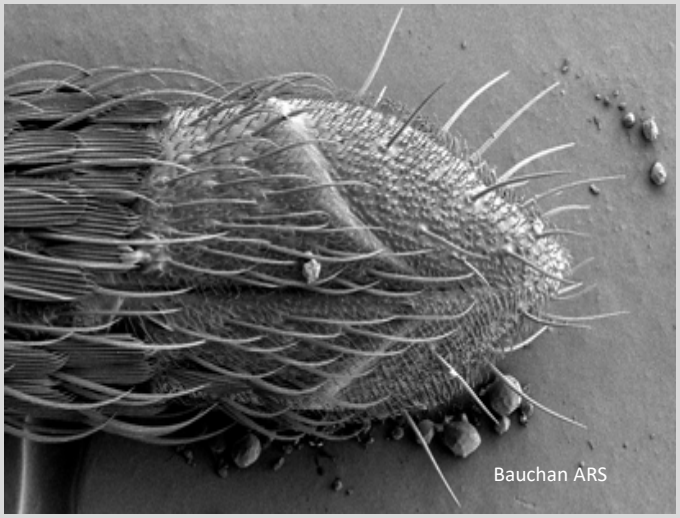
Mosquito Chemoreception of Semiochemicals (1A)

Objective

Determine physiological and molecular mechanisms involved in the detection of attractants, repellents, and feeding deterrents by mosquitoes in order to facilitate discovery of novel behavior-modifying chemicals.

Accomplishment and Impact

- "Single cell" identification of mosquito "taste" receptors.
  Impact: Facilitate screening of mosquito repellents.



Development of New and Optimized Existing Trapping Systems (1A, 1B)

Statement of Problem:

- Effective mosquito/biting fly control requires good surveillance.
- The problem is that “one-size-does-NOT-fit all”.
- Even within a taxonomic group such as *Aedes* mosquitoes, different trap designs and attractant combinations are required in order to properly detect and quantify the presence of a given species.

Accomplishments:

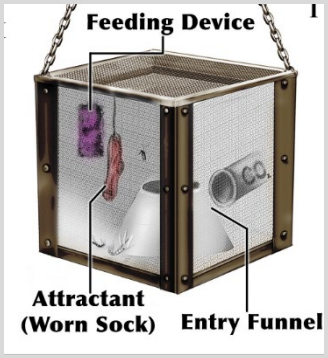
- In collaboration new trap designs, attractants and delivery devices were developed and/or evaluated for mosquitoes and tabanids.

Impact:

- 2 published and 1 accepted manuscripts on new trapping technology.
- 3 more manuscripts in preparation on passive trap design for mosquitoes.
- Tabanid H-trap is staring to be widely used on horse farms.
- Passive mosquito traps are being considered for use in developing countries to assist in studies on malaria epidemiology.

H-trap for Tabanids

Lighting trap for mosquitoes



Passive trap for mosquitoes

Delivery device for attractants

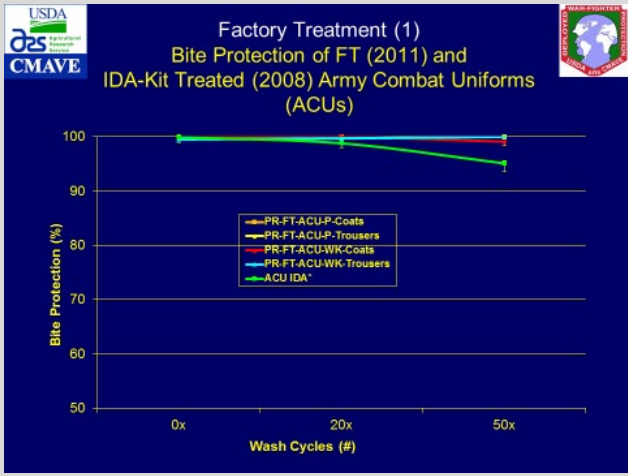
Effective Repellent-Treated Uniforms for the U.S. Military (1A, 1B, 1C, 1D)

Statement of Problem: During nearly all military conflicts, the primary cause of casualty has been disease rather than injuries received directly from conflict. U.S. military personnel that are deployed on foreign soil are often at risk for arthropod-borne diseases, especially when arriving into an endemic areas where vector control programs are non-existent. These personnel often arrive with only their uniform and possibly skin repellents. Therefore, it is important to provide these personnel with the most protective equipment possible.

Bite Protection Assessment of a Permethrin-Treated US Army Fire-Resistant Army Combat Uniform

Accomplishment: From 2007-present, ARS assisted the USMC, then the US Army transition to a supply of combat uniforms that are treated at the factory level. These uniforms retain active ingredient longer than field treatment due to incorporation of a binder. In 2015, ARS conducted the study to register etofenprox as a new clothing repellent.

Impact: The ARS bite protection protocol is now the standard by which US EPA requires efficacy data for registration of insecticide-treated clothing. Etofenprox is the only alternative clothing treatment and will be available to treat undergarments and PT gear, unlike permethrin.



Army Combat Uniforms (ACUs) that are factory-treated with permethrin provide nearly 100% bite protection from mosquitoes through 50 wash cycles.

Passive Vector Control Systems to Protect the U.S. Military (1A, 1B, 1C, 1D)

Problem Statement

Vector control techniques used throughout the world against mosquitoes, sand flies, and filth-breeding flies have been over-generalized from limited trials in a single ecological region (southeast US); fall short of adequately protecting public and veterinary health from nuisance or disease-vector insects.

-

Specific challenge: Military units in remote field positions are exposed to greatest risk of vector-borne disease transmission and nuisance insects, yet are the most underserved by operational pest control.

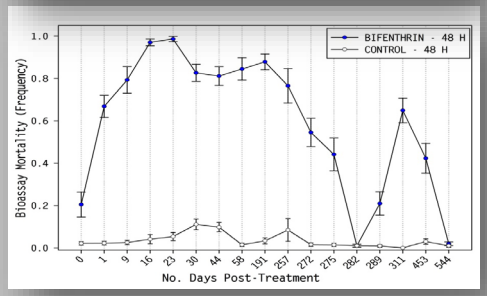
Accomplishment

Demonstrated efficacy of residual pesticide treatments on HESCO geotextile, 4 types of US military camouflage netting, and arid land vegetation, with specific data for 4 formulations and 5 sprayers against mosquitoes, sand flies, and filth flies across warm-temperate (FL, AR), hot-arid (CA, Kenya, Iraq), warm-tropical (Thailand) environments. We developed field-deployable timed pesticide misting system that may operate synergistically with residual treated materials to specifically reduce peak target insect activity and enhance residual treatment.

Impact

Effective passive control to reduce vector and nuisance insect populations may improve and enhance the current DoD pest management system by leveraging existing materials organic to field units and natural resting behavior. Misting system can quickly, safely adjust to respond to specific timing of specific vector threats. Together or independently, these systems can reduce contact between personnel and vector/nuisance insects, commensurately reducing risk of transmission of vector-borne pathogens and disturbance from nuisance species.

Camouflage netting or HESCO enclosures are set up in study habitat with significant biting pressure, treated with residual pesticide, and fitted with a mosquito or sand fly trap baited with CO_2.

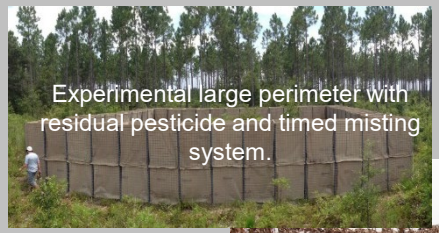


Weeks to months longevity of efficacy indicated in field & lab:

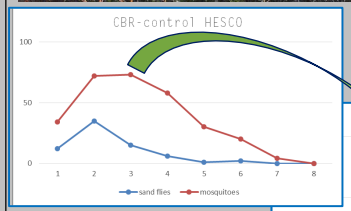
(1) Trap catches in treated enclosures significantly lower compared to untreated enclosures

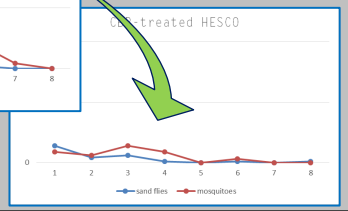
(2) Laboratory bioassays on time series of material samples.

Timed misting system sprays during peak activity; effectively reduces highest-risk biting population.



Experimental large perimeter with residual pesticide and timed misting system.





Real-world OIF scenario for operational use of these systems: presence of camouflage netting and HESCO barriers for residual treatment, forming living area perimeter that may be further protected with timed misting system.

Passive Vector Control Systems to Protect the U.S. Military (1A, 1B, 1C, 1D)

Problem Statement

Vector control techniques used throughout the world against mosquitoes, sand flies, and filth-breeding flies have been over-generalized from limited trials in a single ecological region (southeast US); fall short of adequately protecting public and veterinary health from nuisance or disease-vector insects.

-

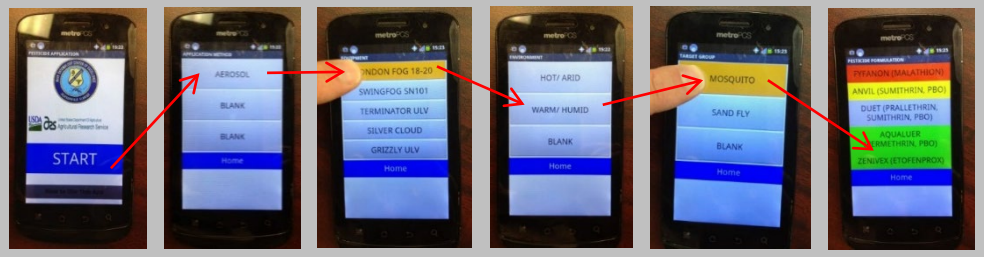
Specific challenge: A gap exists between end-user stakeholders and recent experimentally-derived pesticide delivery methods that are effective, efficient, and sustainable across a range of austere and tropical environments.

Accomplishment

Designed effective and user-friendly front end interface, the mobile Pesticide App to provide specific guidance points to a range of stakeholders, including include military and civilian operational entomology, public health, and pest management professionals, instructors, and researchers. Guidance points derived directly from >10 years of MFRU-led collaborative field, semi-field, and laboratory studies of residual and aerosol pesticide efficacy from diverse formulations, equipment, techniques, target insects, and ecological regions.

Impact

Improved strategy for reduction of adult mosquito, sand fly, and filth fly nuisance and disease-vector populations, leading to reduced disease. From diverse control studies, accumulated >100 operationally relevant guidance points in the form of multiple scientific publications, technical guides, presentations, and unpublished reports that are not synthesized or easily accessed by stakeholders. The mobile device Pesticide App brings together entire portfolio of research into operational pesticide application guidance specific to environment or target insect and easily accessed by stakeholders worldwide.



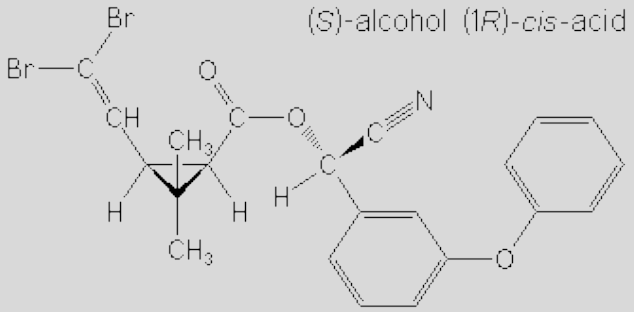
Mobile app – Android platform

Mobile app – iOS platform

Development of New Insecticides, Repellents and Attractants for Arthropod Control (1A, 1B, 1C)

Objective

Design and synthesize novel toxicants, repellents, and attractants that can be used to mitigate the impact of blood-feeding arthropods.



Accomplishments and Impacts

- Developed CO_2 generator not utilizing dry ice.

  Impact: Improved mosquito surveillance during disease monitoring.

- Developed field-stable mosquito attractants.

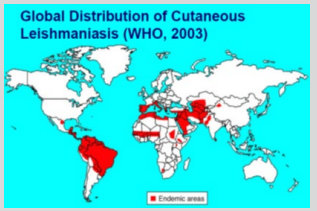
  Impact: Essential for "attract & kill" strategy for vector control, and reduces the risk of insect vectors developing insecticide resistance.

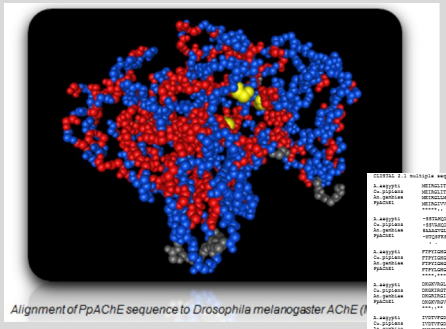
Protection of U.S. Military Personnel from Sand Flies(1C)

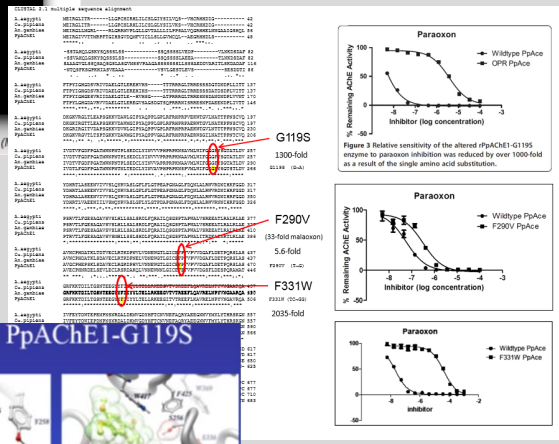
Statement of Problem: The sand fly, Phlebotomus papatasi, is a major vector of cutaneous leishmaniasis in the Middle East and was initially responsible for about 50% of sick days for U.S. military personnel deployed in Iraq and Afghanistan. Over 350 million people are at risk of leishmaniasis and over 2 million new cases are reported each year. There is a substantial need for identification and development of toxicants, repellents and other technology for sand fly control.

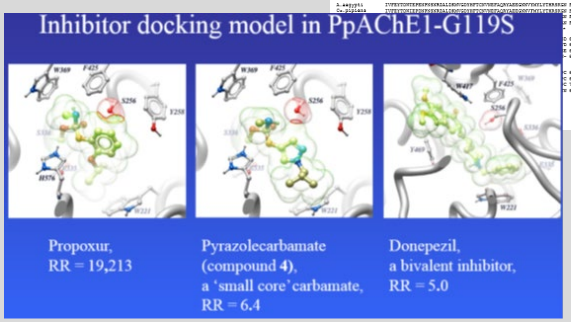
Accomplishment: Acetylcholinesterase of *P. papatasi* (PpAChE1) was sequenced, expressed as an enzymatically active recombinant protein, biochemically characterized, and mutations resulting in resistance to organophosphate (OP) and carbamate insecticides were characterized. Novel synthetic carbamates were identified that exhibited over 500-fold improved safety for mammalian vs. arthropod AChE inhibition, and additional synthetic carbamates were effective inhibitors of OP-resistant PpAChE1 recombinant enzymes.

Impact: Molecular mechanisms of OP resistance were characterized and novel synthetic carbamates were identified with improved mammalian safety or efficacy against OP-resistant arthropod AChEs.




Alignment of PpAChE sequence to Drosophila melanogaster AChE (





Development of New Tools and Strategies for Medically-Important Ticks (1D)

Objective

Discover and develop new tools for the control of ticks affecting humans, and evaluate their effectiveness at a range of conditions associated with climate change.

Accomplishments and Impacts

- Developed artificial feeding *system for lone star ticks.*
  Impact: Eliminate need for animal/human hosts for testing.

- Determined no correlation in repellency based on infection status of deer ticks.
  Impact: Use of tick repellents are still justified to reduce Lyme disease transmission.

Improved Surveillance and Control of Bed Bugs(1E)

Objective

Develop improved surveillance and control techniques for bed bugs.

Accomplishments and Impacts

Terzi (from Usinger 1966)

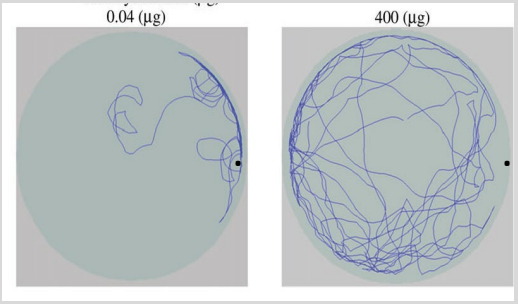
➢Determined that adult male bed bugs can be used exclusively in laboratory bioassays, thereby preserving female bed bugs for colony purposes.

Impact: Easier for industry and academia to develop new chemicals to mitigate bed bug infestations by insuring sufficient biological material for laboratory testing of pesticides.


American Pest

➢Identified the bed bug-derived compounds that canines detect.

Impact: Less expensive than buying bed bugs for training.



➢Determined that bed bug “defensive” secretions can “attract” at low levels.

Impact: Develop new detections tools.

Questions on Component 1?

Component 2: Veterinary entomology

Problem Statement 2A: Invasive ticks

Problem Statement 2B: Stable flies

Problem Statement 2C: House flies

Problem Statement 2D: Horn flies

Problem Statement 2E: Screwworm flies

Problem Statement 2F: Mosquitoes

Problem Statement 2G: Biting midges

Anti-Cattle Tick Vaccine Antigens for Large Animal Trials (2A)

Statement of Problem: The USDA-APHIS Cattle Fever Tick Eradication program needs novel control technology options that are effective against cattle fever ticks. An existing anti-cattle tick vaccine has variable efficacy and new vaccines are needed.

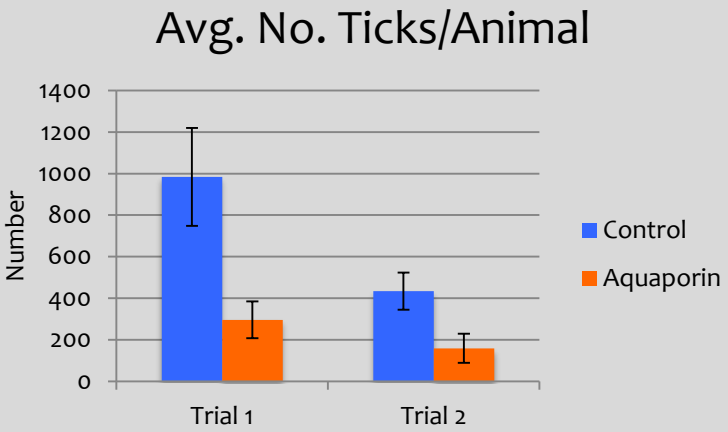
Accomplishment: Utilizing cattle tick genomic data, ARS scientists have produced 7 novel anti-cattle tick vaccine antigens scheduled for evaluation by CRADA partners. Ten patent applications with US and foreign Patent Offices have resulted from our vaccine discovery research.

Impact: Anti-cattle tick vaccine technology has the potential for controlling the cattle tick and reducing the incidence of bovine babesiosis. These are significant problems worldwide.

Key Points:

Evaluation of ARS Ag1 vaccine by Brazil collaborator

- Groups of 6 Holstein calves
- 3 doses spaced 3 weeks apart
- Monitored Antibody response for 4 months
- Assayed yield of engorged female ticks, eggs, and egg hatch
- 72% efficacy in cattle tick control

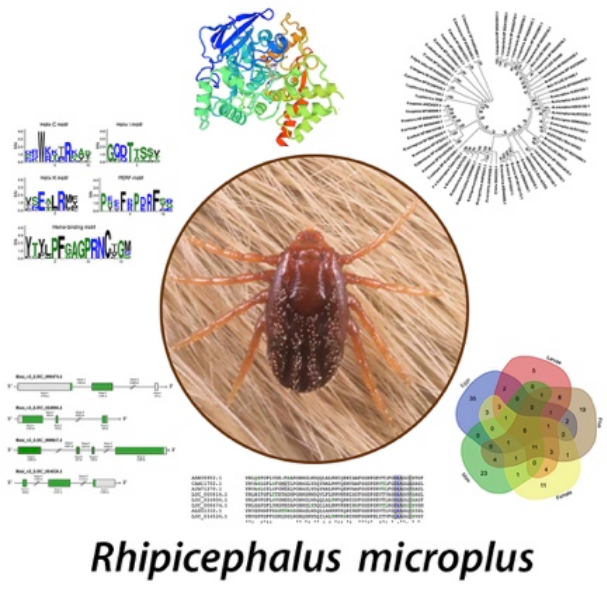


Sequencing and Assembling the Cattle Fever Tick Genome (2A)

Statement of Problem: The USDA-APHIS Cattle Fever Tick Eradication program's needs novel control technologies effective against cattle fever ticks. The genome, which can guide development of these novel technologies, is huge and complex to sequence and assemble.

Accomplishment: Collaborating with NCGR and Murdoch University sequencing and bioinformatics experts, ARS scientists sequenced and assembled the genome of the cattle fever tick and identified the full complement of genes.

Impact: The data has been published and released to the scientific community. This new comprehensive dataset is facilitating anti-tick vaccine research and pesticide resistance monitoring.



Key Points: Genome has allowed identification of

- Genes causing pesticide resistance.
- Anti-tick vaccines targets
- Evolutionary pathways of cattle tick's global speciation

Translational Research to Control Cattle Fever Ticks Infesting Exotic Nilgai Antelope (2A)

Problem

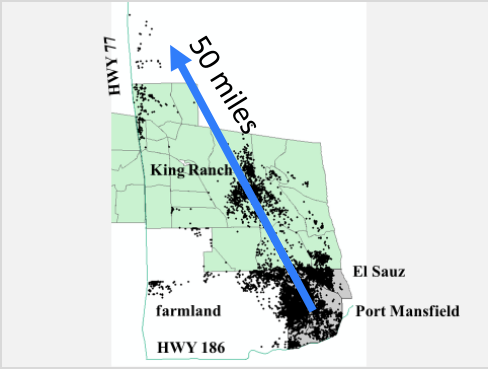
- Exotic nilgai antelope (*Boselaphus tragocamelus*) are competent hosts of cattle fever ticks (CFT), and are implicated in the long-range spread of CFT beyond the Texas-Mexico border.
- The home range of nilgai remains to be fully understood, which complicates the limits of tick quarantines.
- There were no known lures for, or methods to treat CFT infestations in nilgai, including the treatment of, and surveillance for infested nilgai in environmentally sensitive wildlife refuges

Results

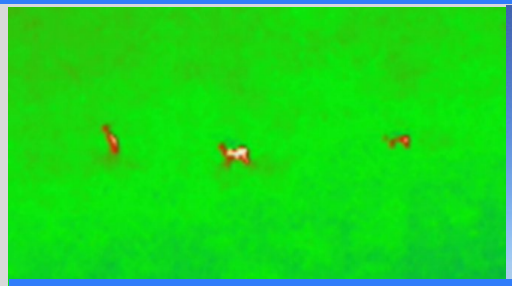
- Nilgai with satellite collars moved more than 50 miles from El Sauz to the King Ranch. Young females have the largest home range at > 40,000 acres.
- Screwworm lure and offal were found to attract nilgai and induce latrine formation.
- Entomopathogenic nematodes were evaluated for efficacy against CFT. Nematodes are commercially produced and native to Texas, so they meet environmental requirements for use on US. Fish and Wildlife refuges in South Texas.
- Remotely activated 'nilgai sprayers' were developed to treat them as they visit common latrine sites.

Impact

- Cattle Fever Tick Eradication Program (CFTEP) quarantine rules were adapted to reflect the risk of nilgai dispersing CFT.
- Infrared drones more accurately determined the nilgai density per acre in areas with deep brush where they were not visible.
- Nilgai sprayers are being deployed by the CFTEP at sites with CFT-infested nilgai.



Nilgai home range in South Texas. Nilgai at screwworm lure site

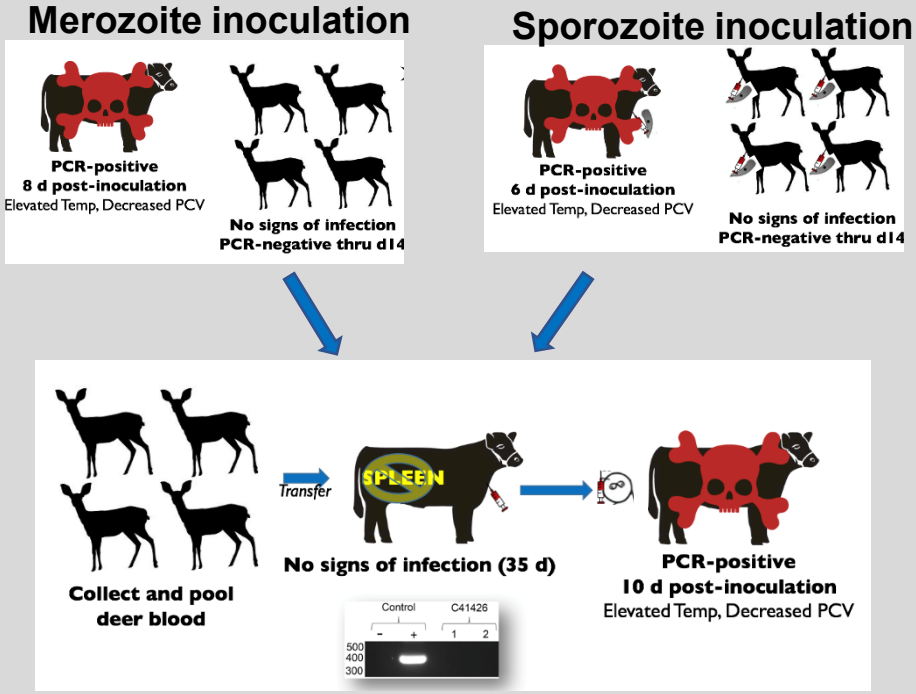


Nilgai detected with infrared drone. Infrared FLIR drone

Field sprayer set up on nilgai latrine. Tick killing nematode *Steinernema riobrave*.

White-tailed Deer are Hosts for Cattle Fever Ticks but not a Reservoir of Bovine Babesiosis Agent (2A)

Statement of Problem: White-tailed deer and exotic ungulates and bovids are implicated in the spread of cattle fever ticks throughout southern Texas, threatening the federally funded Cattle Fever Tick Eradication Program. Current economic trends have resulted in land diversification in Texas that increases overlapping habitat between cattle and white-tailed deer. Since cattle fever ticks feed on cattle and deer indiscriminately, it is critical to understand whether deer are reservoirs for tick-transmitted bovine diseases.



Accomplishment: Demonstrated that white-tailed deer are not susceptible to *Babesia bovis* infection, a study in collaboration with ARS NP-103 scientists.

Impact: Understanding the role that wildlife play as reservoirs of bovine pathogens is important to the US cattle industry. Surveillance for these pathogens in other wildlife that can serve as hosts for cattle fever ticks is critical, and experimental infection studies inform molecular and serological assays used in surveillance.

Mitigation of Pathogenic Landscape Forming Weeds that Facilitate Cattle Fever Tick Invasion (2A)

Problem: *Arundo donax*, or giant reed, is an exotic and invasive weed of riparian habitats in the southwestern U.S. and northern Mexico. *Arundo* dominates these habitats, which leads to: 1) creation of pathogenic landscape by altering microclimate and reducing populations of ground-dwelling tick predator insects, which increases the risk of cattle fever tick (CFT) incursion; 2) loss of biodiversity; 3) stream bank erosion; 4) reduced visibility of the international border for the U.S. Border Patrol; 5) competition for water resources in an arid region where these resources are critical to the environment, agriculture, and urban users. Biological control using specialist insect agents from the native range of *A. donax* in Europe may be the best option for long-term and widespread management of this weed.

Results: Biological control insects from the native range of *Arundo* in Spain were released and became established in Texas & Mexico. Along 500 miles of the Rio Grande from Del Rio to Brownsville, *Arundo* biomass was reduced by 32%, the canopy opened to light penetration, within-stand visibility increased from 9 to 27 ft from 2009 to 2017 pre- and post-release of the biological control agents, and desirable native riverine vegetation is regrowing which leads to return of tick predator insects in the Cattle Fever Tick Permanent Quarantine Zone.

Impacts. The biological control program: 1) re-established a biological barrier to CFT invasion mitigating the effects of the pathogenic landscape; 2) documented Rio Grande Basin-wide water conservation by saving 6000 acre feet of irrigation water per year valued at $4.4 million annually; and, 3) increased visibility of the international border, which enhanced National Security.

Arundo donax, along Rio Grande in Texas

Plant-feeding arundo wasp ovipositing in arundo stem. Damage to weed by the biological control insect leads to regrowth of desirable native vegetation that supports tick predators, conserves water, and increases visibility for U.S. Border Patrol.

Ecologically-based Approaches Addressing the Cattle-Wildlife Interface Impacting Cattle Fever Tick Eradication Program Operations (2A)

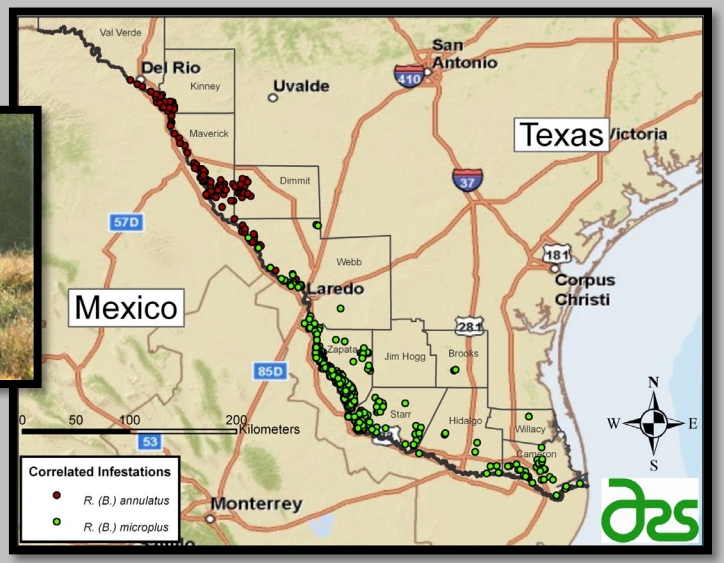
Statement of Problem: Novel, efficacious tools are needed to monitor and control cattle fever tick infestations on cattle and wildlife caused by *Rhipicephalus microplus* and *R. annulatus* in the context of landscape changes impacting operations of the Cattle Fever Eradication Program (CFTEP) managed by APHIS-Veterinary Services and the Texas Animal Health Comission.

Accomplishments: 1) Development of the '2-poster' deer treatment station; 2) Development and maintenance of a historic GIS database that tracks cattle fever tick outbreaks in south Texas spanning decades.

Impact: 1) The '2-poster' deer treatment stations are routinely used by the CFTEP to treat cattle fever ticks on deer in support of efforts to eliminate tick outbreaks; 2) The historic GIS database is utilized by the CFTEP on a daily basis to build maps and develop other predictive tools that are used to manage the program.

Key Points:

The '2-poster' device effectively treats cattle fever ticks infesting white-tailed deer.



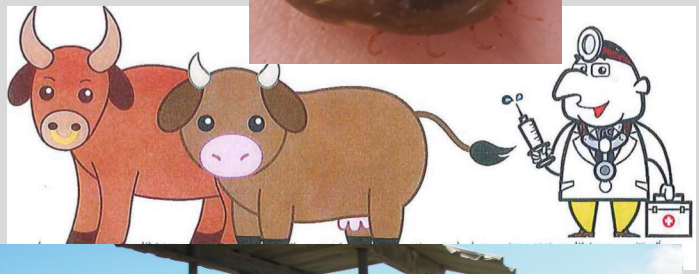
The historic GIS database was used to create the map shown above depicting the distribution of cattle fever tick infestations in south Texas between 1999 and 2010.

Advancing Areawide Cattle Fever Tick Management (2A)

Problem: Initial attempts to eradicate, & then efforts to control the southern cattle fever tick (SCFT), *Rhipicephalus microplus*, in Puerto Rico (PR) were unsuccessful. By 2013, outbreaks of the SCFT-borne diseases bovine babesiosis and anaplasmosis were causing losses of ~$29 million to the local dairy cattle industry.

Accomplishment: Working with livestock producers, and state and federal veterinary services agencies, ARS led a 3-year research project for integrated control of the SCFT in PR. The project was completed successfully before hurricane Maria ravaged PR.

Impact: Areawide management was practiced to implement integrated SCFT control protocols. Research outcomes mitigated food safety and environmental health risks related to previous intense use of chemicals to control SCFT infestations in lactating dairy cows. Mortality due to bovine babesiosis and anaplasmosis was abolished in the dairy and beef farms involved in the research project. The safe use of an anti-SCFT vaccine in lactating dairy cows was demonstrated. Livestock producers asked the local government to implement an areawide SCFT control program in PR based on the results of this research project.



Integrated Use of Anti-Cattle Fever Tick Vaccine (2A)

Problem: Despite the successful efforts accomplished by the Cattle Fever Tick Eradication Program (CFTEP) in 1943, cattle fever ticks (CFT) threaten the U.S. livestock industry because they are vectors of the deadly disease bovine babesiosis and are established in our neighboring country to the south. New technologies that can be integrated with existing protocols are required for sustainable CFTEP operations.

Accomplishment: Through its anti-tick vaccine discovery research program, ARS led the establishment of a public-private partnership that obtained an experimental use permit from the Center for Veterinary Biologics for an anti-CFT vaccine. The CFTEP is using this technology.

Impact: Statutes governing CFTEP operations, which are more than 100 years old, were adapted to integrate the use of the anti-CFT vaccine. This is a favorable outcome for the CFTEP. The anti-CFT vaccine was also used in Puerto Rico in an areawide CFT management research project to protect dairy & beef cattle against CFT infestations.

NEWS RELEASE

Texas Animal Health Commission

"Serving Texas Animal Agriculture Since 1893"

Andy Schwartz, DVM • Executive Director

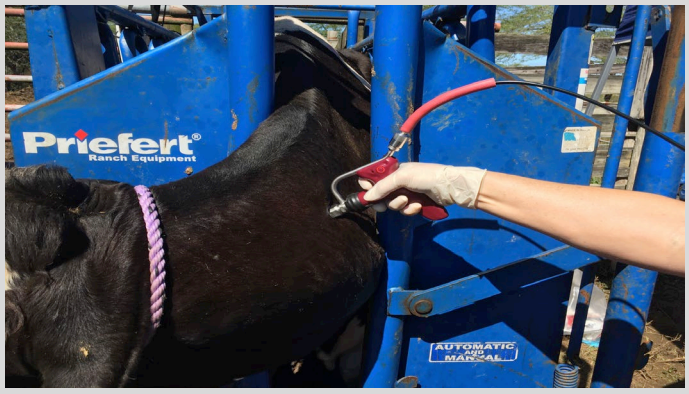
P.O. Box 12966 • Austin, Texas 78711 • (800) 550-8242 http://www.tahc.texas.gov

For more information contact the Communications Dept. at 512-719-0750 or at callie.ward@tahc.texas.gov

June 7, 2016

Tackling the Cattle Fever Tick with Vaccine

AUSTIN – The Texas Animal Health Commission (TAHC) is proud to announce the arrival of a new tool in fever tick eradication efforts. The new fever tick vaccine will be a valuable tool for reducing the risk of new fever tick infestations in quarantine areas such as the tick eradication quarantine area, or permanent quarantine zone, and in temporary preventative or control quarantine areas.



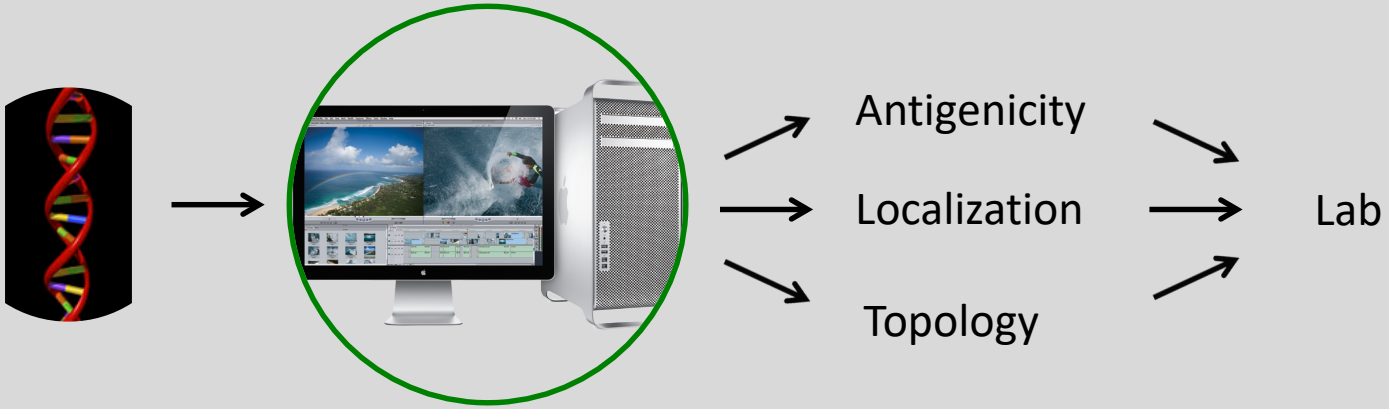
Bioinformatic Prediction of Vaccine Antigen Candidates (2A, 2D)

Statement of Problem: The proteomes of the cattle tick and horn fly are very large, presenting > 40,000 potential targets for vaccine-based control technology. A science-based means to prescreen the proteome is needed to bring the number of targets for individual study to a manageable value.

Accomplishment: ARS scientists have implemented and adapted a set of computational algorithms into a bioinformatic pipeline for Reverse Vaccinology. This has been implemented for genome-driven anti-cattle tick and anti-fly vaccine research.

Impact: This Reverse Vaccinology bioinformatic pipeline has been used to select anti-cattle tick and anti-fly vaccine antigens, removing a significant uncertainty in choosing proteins to produce and evaluate for control efficacy in large animal studies.

Key Points:



Economic Impact of Stable Flies on Cattle & Dairy Production (2B)

Statement of Problem: Accurate estimates of economic impact are essential for developing IPM programs and prioritization of research & development needs. Such information for stable flies was outdated and lacked quantitative methods.

Accomplishment: A model of the economic impact based upon commodity values, cattle inventories, and stable fly infestation levels was developed. The model estimates annual production losses in the US to be over $2 billon per year.

Impact: Results indicate stable fly is the most damaging arthropod pest of cattle in the US. Producers and policy makers can quantitatively assess the cost-benefits of stable fly management and research.

Pastured cattle grazing without stable flies.

Stable flies digesting their bloodmeal in a dairy.

Stable flies biting and the results.

Biology of Immature Stable Flies (2B)

Statement of Problem: Although control technologies are most effective when applied towards immature stages, many questions remain relative to the relationships between stable fly larvae and their developmental substrates.

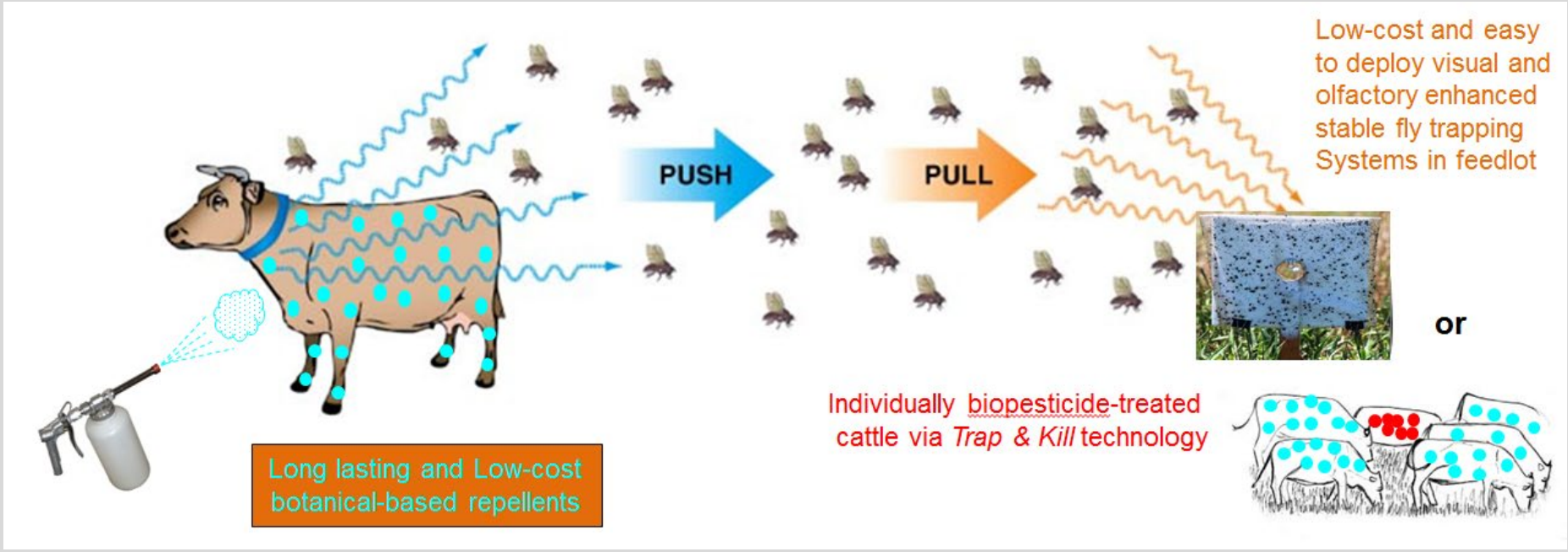
Accomplishments:

- Identified chemical attractants and repellants for stable fly larvae
- Described the ultrastructure of stable fly and house fly larvae including sensory organs
- Modeled relationships of temperature and diet quality on size and developmental rate of immature stable flies
- Characterized substrate and symbiotic microbial communities
- Described chemical and physical properties of larval substrates
- Developed aseptic method for rearing stable fly larvae

Impact:

- Advanced understanding of how stable fly larvae develop and interact with their environment

Push-Pull Strategy to Manage Biting Flies on Cattle (2B)



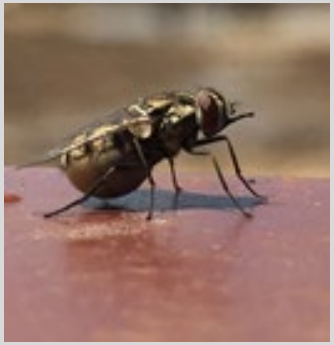
Problem

- **The infestation of stable flies caused US livestock industry and producers > $2 billion dollars of losses**
- **Very limited research have been conducted to deal with these problems**

Results and Impact

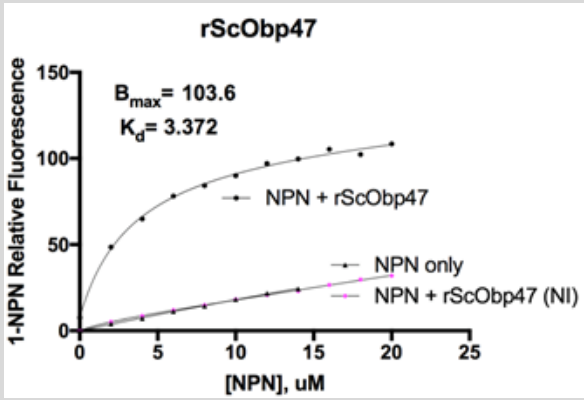
- **Demonstrated olfactory and visual cues are two primary tools for their host and oviposition site seeking**
  - Development of cost-effective visual and olfactory enhanced trapping systems (10-times less costs compared to traditional ones)
- **Discovery and development of long-lasting and low-cost botanically based repellents and biopesticides against biting flies.**
  - First repellent compound identified from catnip oil with over 95% of repellency (spatial and contact), as well as a oviposition deterrent
  - A long-lasting biting fly feeding deterrent identified from coconut oil providing over two weeks long effectiveness (lab condition) and 1-week longevity under the field condition (with the developed starch-based formulation). The longest repellent discovered so-far.
  - The cost of each application materials around $0.08 per animal (whole body), significantly lower than any commercial pesticides
  - Also repel serious disease-transmitted other biting insects, such as ticks and bed bugs (patent filed)

Completion of Stable Fly Genome Sequence (2B)

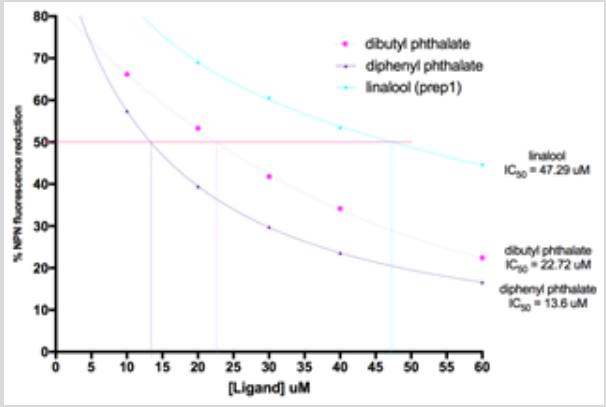


Statement of Problem: Stable flies are responsible for an estimated 2 billion USD damage annually to the U.S. cattle industry. The flies are a nuisance to people and animals, capable of inflicting a painful bite. There are large knowledge gaps related to stable fly biology, and a need for development of new methods for adult control and bite prevention.

Accomplishment: The stable fly genome was sequenced and annotated in collaboration with Yale University, the University of Cincinnati, and members of the stable fly genome consortium. Gene family expansions occur in chemosensory, vision, immune system, and metabolic detoxification pathway genes. Recombinantly produced stable fly odorant binding proteins (OBPs) facilitated assays for ligand binding efficiencies. New studies characterizing stable fly repellents and toxicants were completed. MicroRNAs of stable flies were identified and sequenced.



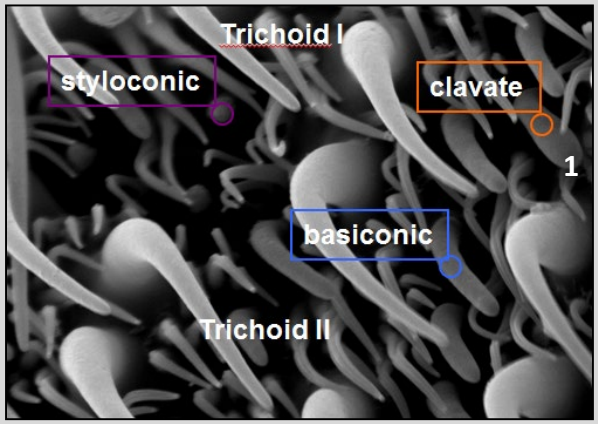
Impact: Availability of the stable fly genome enables data mining to identify targets for development of novel control technologies targeting stable fly feeding and reproduction. Recombinant OBPs provide a mechanism for high throughput screening of chemical libraries to identify lead repellents and attractants. Identification of new toxicants and repellents will improve stable fly control and bite reduction.

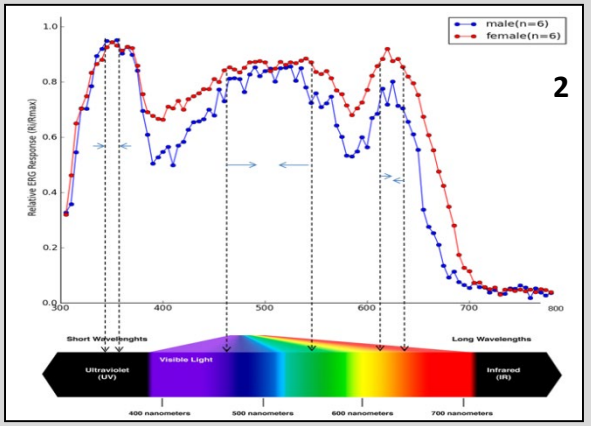


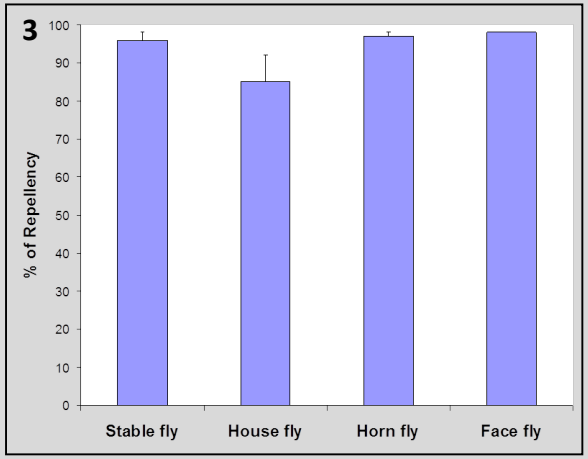
Discovery of a Biting Fly and Tick Repellent (2B,2A)

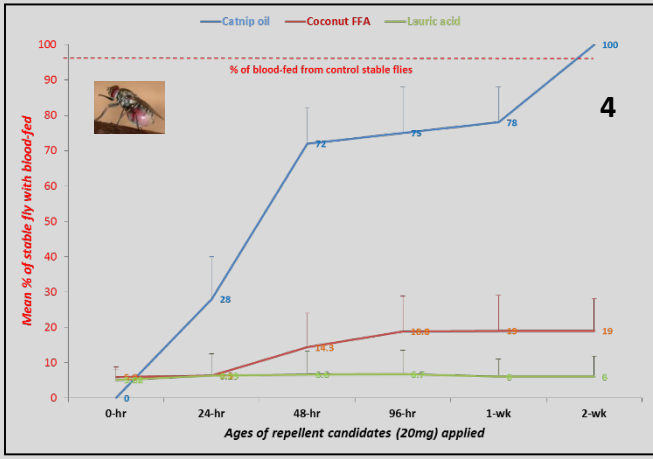
Accomplishment/Impact:

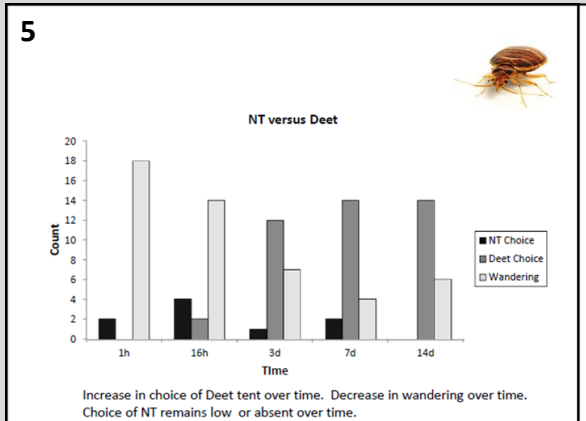
1. Olfactory sensilla with 3-D distribution on antennae
2. First electroretinogram recording completed on stable fly
3. A universal fly repellent identified from catnip oil
4. A long-lasting biting fly feeding deterrent identified from coconut oil with 2-week effectiveness discovered
5. The long-lasting repellent fatty acids from coconut oil strongly repel bedbugs, with 2-week effectiveness, while DEET only provides 16 hours' efficiency
6. The patented coconut fatty acid repellent also effectively repelled various disease-transmitted tick species, with the lowest concentration observed at 0.3%

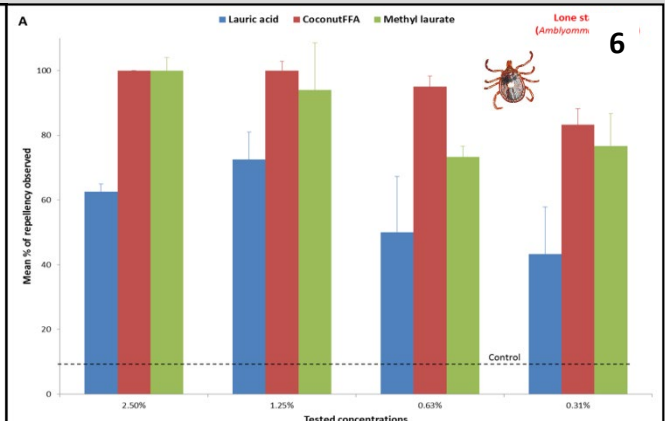












Development of Traps to Capture Stable Flies (2B)

Statement of Problem: Blue and black cloth targets treated with a pesticide were developed in Africa for tsetse fly management. In the US, where targets were adapted for stable fly management, the flat 1 meter square design posed problems in great plains states where targets might be affected by high winds. The large size was also a problem because of difficulty in handling and cost of fabrication materials.

Accomplishment: In a 3-year study target shape and size were compared with the original flat design. Cylindrical targets better withstood winds without a reduction in attraction to flies, and smaller cylindrical targets attracted stable flies in numbers similar to the large targets. Finally, black cloth alone was as attractive as the blue black combination.

Impact: This research demonstrated that a useful tool for African tsetse flies could be modified and improved for use against stable flies in the US. Use of a single color instead of two reduces fabrication costs and smaller size increases ease of handling. This is a device that producers can make themselves and place in areas where flies are a problem.

Efficient Trap for Stable Flies (2B)

Statement of Problem: The stable fly is a major livestock pest in the US and currently available pesticide-free sticky traps were not efficient at all temperature ranges and were difficult to move to new trapping sites. Also, trapping techniques required that traps be placed out of the reach of animals to prevent animals from destroying the traps. Traps placed close to animals capture more flies.

Accomplishment: The Knight Stick Sticky trap was new on the market and significantly outperformed standard sticky traps. The sticky wraps placed around the trap to capture the flies reflect sunlight in wavelengths attractive to the flies. Sticky wraps attract flies when used on the traps and when wrapped around other cylindrical objects. To overcome trap placement problems, Knight Stick traps were placed inside of square enclosure made from electric fence and placed in exhibit yards with the animals.

Impact: The Knight Stick trap captured at least 3 times the numbers of flies captured with sticky traps currently in use. The Knight Stick trap is easy to use, has a small foot print, and is easy to move from one place to another as needed. Placing Knight Stick traps inside of electric fence enclosures allowed for placement of traps very close to animals. This increased the fly catch by 6 to 9 times and increased animal comfort and welfare by reducing the painful bites from the flies. The animals were accustomed to electric fence and we never observed them contacting it.

Modeling Stable Fly Population Dynamics Relative to Weather (2B)

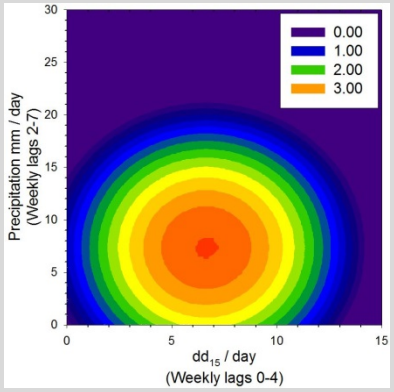
Statement of Problem: The effects of weather and climate on stable fly populations are unclear making predictions of population trends unreliable.

Accomplishment: Analysis of 13 years data from 25 stable fly traps revealed weather variables accounted for 72% of the observed fluctuations. Maximum populations were observed with an average daily temperature of 21°C and 6 mm precipitation / day.

Impact: The model allows producers to consider predicted stable fly population trends when evaluating management options. This reduces the amount of insecticide in the environment and the potential for the development of insecticide resistance.

Thirteen years of stable fly catch data from 25 traps located near Ithaca, Nebraska.

Broce alsynite trap.



Optimal conditions for stable fly population growth were 21°C and 6 mm precipitation / day.

Insect Growth Regulators for Control of Stable Fly Larvae (2B)

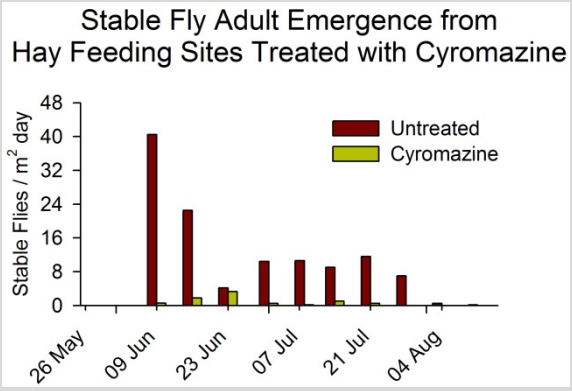
Statement of Problem: Methods for controlling the development of immature stable flies in animal wastes are inadequate.

Accomplishment: Two Insect Growth Regulators, Cyromazine and Novaluron, with two distinct modes of action reduce the number of adult stable flies emerging from winter hay feeding sites by >90%. One treatment effective for entire season.

Impact: First practical method for controlling immature stable flies in biologically active substrates. Currently being used on ≈20,000 ha of pineapple residues in Costa Rica per year.

Granular and liquid formulations easily applied.

Efficacy assessed with emergence traps.



>90% control for entire season.

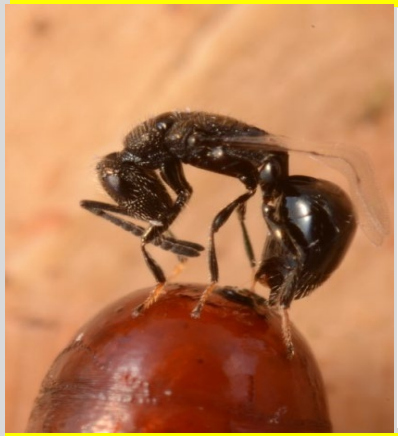
Insect Growth Regulator For Control of House Flies (2C)

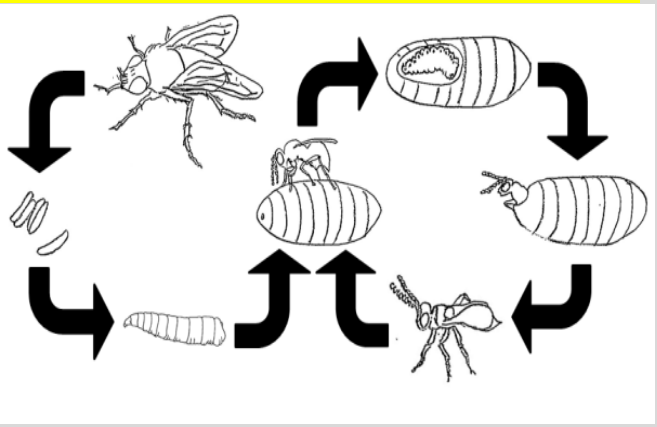
Statement of Problem: New tools are needed for management of house flies because of resistance to nearly every insecticide used for their management. Pyriproxyfen (PPF) is an IGR with good potential but its efficacy and compatibility with natural enemies is unknown.

Accomplishment: House flies were demonstrated to be ca. 1000 times more susceptible to pyriproxyfen than their four principal natural enemies. Surveys of US and Israeli fly populations found little evidence for tolerance in wild flies from any location.

Impact: PPF can be pursued as an alternative fly management tool. Work is continuing to develop autodissemination devices to self-treat gravid female flies and to determine the % of flies that must be treated to give satisfactory control.

Pyriproxyfen is a juvenile hormone analog that prevents insects from completing development from the pupal to adult stages.





Pupal parasitoids are important natural enemies of flies, and any new fly control method needs to take them into account.

Susceptibility of house flies to pyriproxyfen when fly eggs were added to treated larval diet or manure from dairy and beef cattle.

Substrate	LC 90 (95% FL)
Lab media	0.265 (0.16-0.56)
Dairy manure	4.119 (3.40-5.28)
Beef manure	17.628 (14.75-21.7)

Susceptibility of house fly parasitoids to pyriproxyfen.

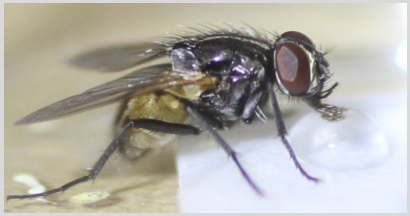
Species	LC 90 (95% FL)
Muscidifurax raptor	> 6000 ppm
Muscidifurax zaraptor	> 6000 ppm
Spalangia cameroni	> 6000 ppm
Spalangia endius	> 6000 ppm

Factors Affecting Survival of Bacteria in Adult House Flies (2C)

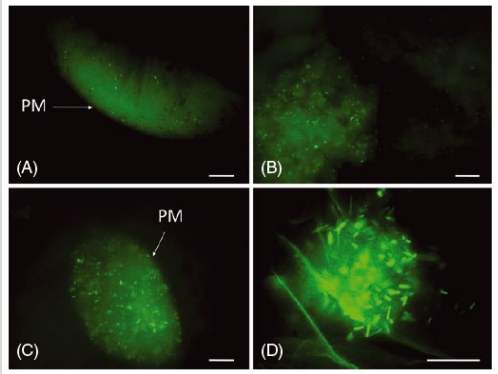
Problem: House flies develop within and feed upon septic substrates teeming with microbes. Being highly mobile and gregarious, adult flies serve as a bridge between filth and sanitized areas such as domestic settings. On farms, flies disseminate microbes from larval developmental sites (e.g. manure piles) to humans, livestock and their food. Thus, from public health, animal production and food safety perspectives, flies are important reservoirs and disseminators of pathogens. Pathogen transmission requires persistence in the house fly gut, and is further bolstered by proliferation. However, the factors impacting pathogen "fate" have not been well studied.

Accomplishments: In a series of experiments, ARS determined that properties of bacteria, including species and abundance, affect their "fate" (persistence, proliferation) in the alimentary canal. Some species, such as *Pseudomonas* and *Salmonella,* persisted and proliferated in the fly gut while others like *E. coli* were rapidly immobilized and slowly destroyed by lysis. Bacterial abundance, or the "dose" ingested by flies, also impacted survival dynamics in the gut. While bacteria in high abundance were lysed in the fly gut, large numbers still survived indicating potential transmission. However a greater proportion of bacterial populations persisted in flies that ingested low doses.

Impact: When bacteria are ingested by house flies, both species and abundance are integral in vector potential and must be considered when assessing risk of pathogen transmission and dissemination.

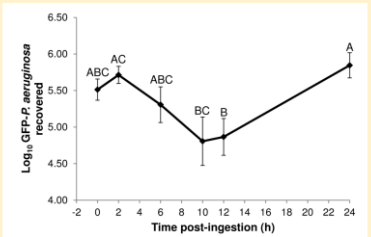


House fly, *Musca domestica,* feeding on bacteria (top). GFP-expressing bacteria used in fly feeding assays (right).

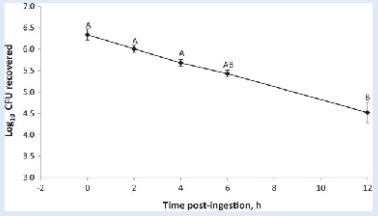


Species-specific bacterial persistence

Pseudomonas aeruginosa



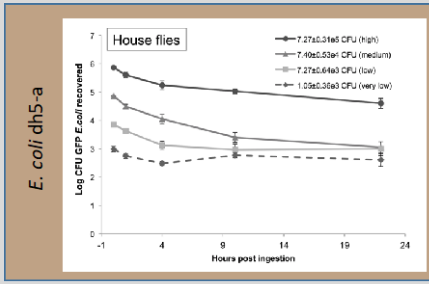
E. coli O157:H7



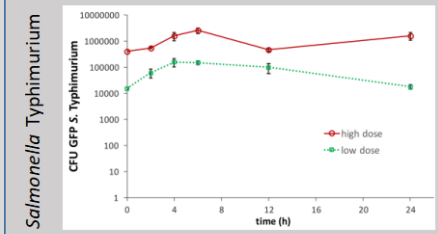
Pathogenic bacteria have unique interactions with house flies in the alimentary canal that result in species-specific impacts on persistence. *Pseudomonas* both persisted and proliferated in the gut of house flies while *E. coli* O157:H7 abundance declined steadily over time. Microscopy (not shown) showed that *E. coli* were immobilized and lysed, while *Pseudomonas* remained highly viable, which may have afforded them an opportunity to evade house fly digestion and defense.

Dose-dependent bacterial persistence

E. coli dh5-a



Salmonella Typhimurium



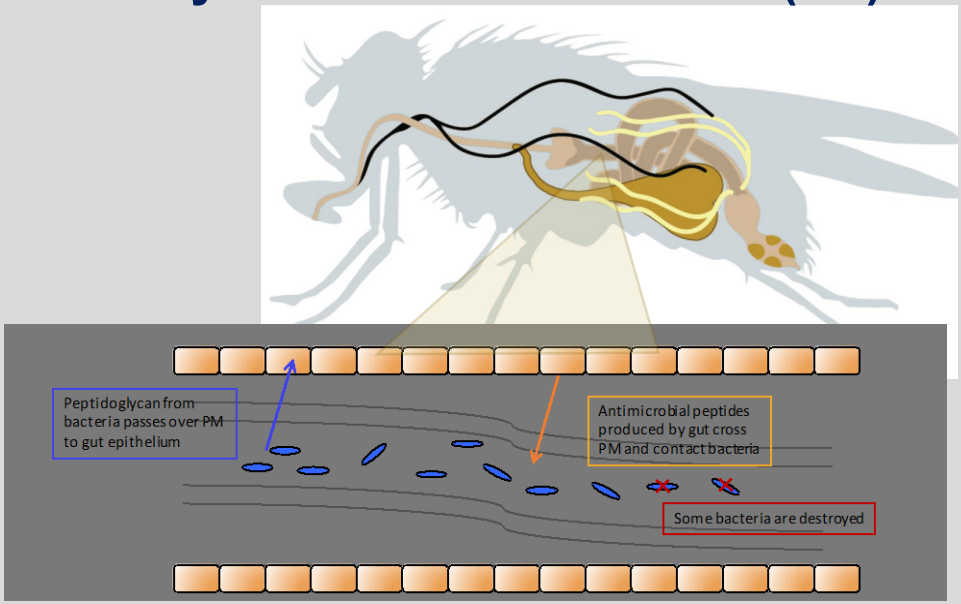
Both *E. coli* and *Salmonella* demonstrated dose-dependent survival in the house fly gut. Although a greater abundance of bacteria survive in flies that ingested high doses, a greater proportion of the ingested bacteria survive in flies fed lower doses. Thus, flies ingesting high-doses are higher-risk vectors, and flies ingesting low-doses may be longer-term disseminators. Further, the proliferation and persistence of *Salmonella* in flies indicates high vectoring potential for this pathogen.

Role of House fly Immune Response in Fly-Bacteria Interactions (2C)

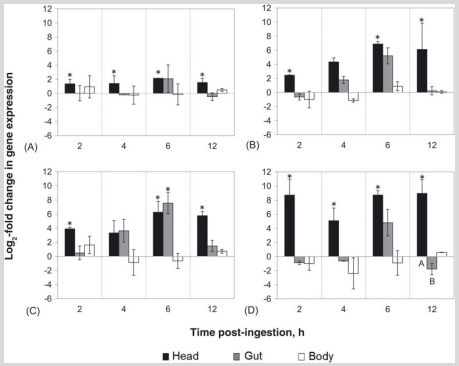
Problem: When house flies breed in environments such as garbage or manure they acquire numerous species of bacteria, many of which are pathogenic to humans, livestock and even other insects. Remarkably, house flies largely are unharmed by these transient microbial residents, even when bacteria are ingested. The mechanism by which flies protect themselves from bacteria has been understudied. Because fly defenses impact bacterial survival, they ultimately are important in vector competence and transmission potential.

Accomplishments: ARS identified genes and transcripts for antimicrobial peptides; effector molecules associated with the house fly immune responses that lyse and kill bacteria. The expression (mRNA, protein) of these genes was determined in response to adult fly ingestion of different species of pathogenic bacteria. Flies mount an immune response to ingested bacteria, indicating that some of these traditional immune genes have been co-opted for a digestive function in the gut.

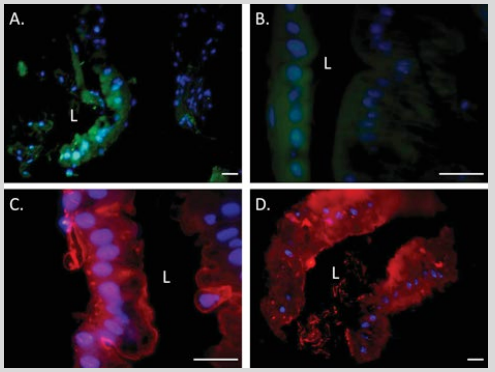
Impact: Understanding how the fly immune system either promotes or hinders the fate of bacteria within flies can help us identify new points for interceding in the spread of fly-transmitted pathogens to both humans and livestock. Additionally, characterizing antimicrobial peptides and other immune effectors in house flies presents an opportunity to explore potential alternatives to traditional antibiotic therapies.



Antimicrobial peptide gene expression in house flies fed *E. coli* O157:H7.



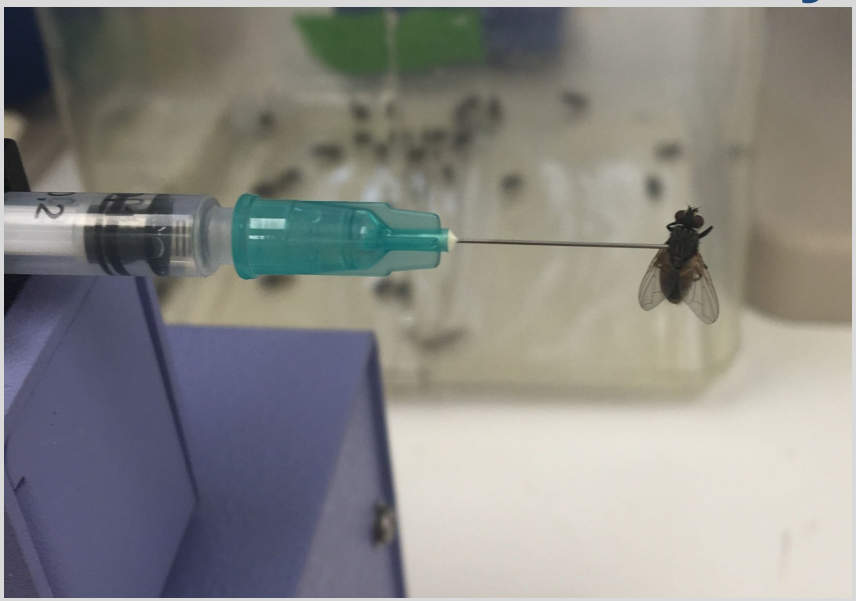
qRT-PCR detection of mRNA expression of (A) cecropin, (B) defensin, (C) diptericin and (D) lysozyme in the head (including salivary glands) and body in response to bacterial peptidoglycan in the gut.



Expression of antimicrobial peptide proteins in sections of the house fly midgut after ingesting *E. coli* O157:H7. (A) diptericin, (B) control, (C and D) lysozyme (2 and 6 h post feeding).

House flies mount an immune response to bacteria in the alimentary canal, showing a co-option where immune defense genes are used locally for digesting bacteria.

Pathogen Combination Leads to More Rapid House Fly Mortality (2C)



Statement of Problem: The fungal pathogen *Beauveria bassiana* is efficient at infecting house flies but takes 6 days to kill them. Can the death rate be sped up by combining it with a second, bacterial pathogen?

Accomplishment: Three bacterial pathogens were tested with *B. bassiana* after screening for virulence by injection: *Photorhabdus temperata*, *Serratia marcescens*, and *Pseudomonas protegens*. A nonionic surfactant was identified (CapSil 0.5%) that is safe for topical application and promotes survival of bacterial cells and fungal conidia. Topical treatment with combinations of *B. bassiana* and *P. protegens* killed flies much faster than *B. bassiana* alone.

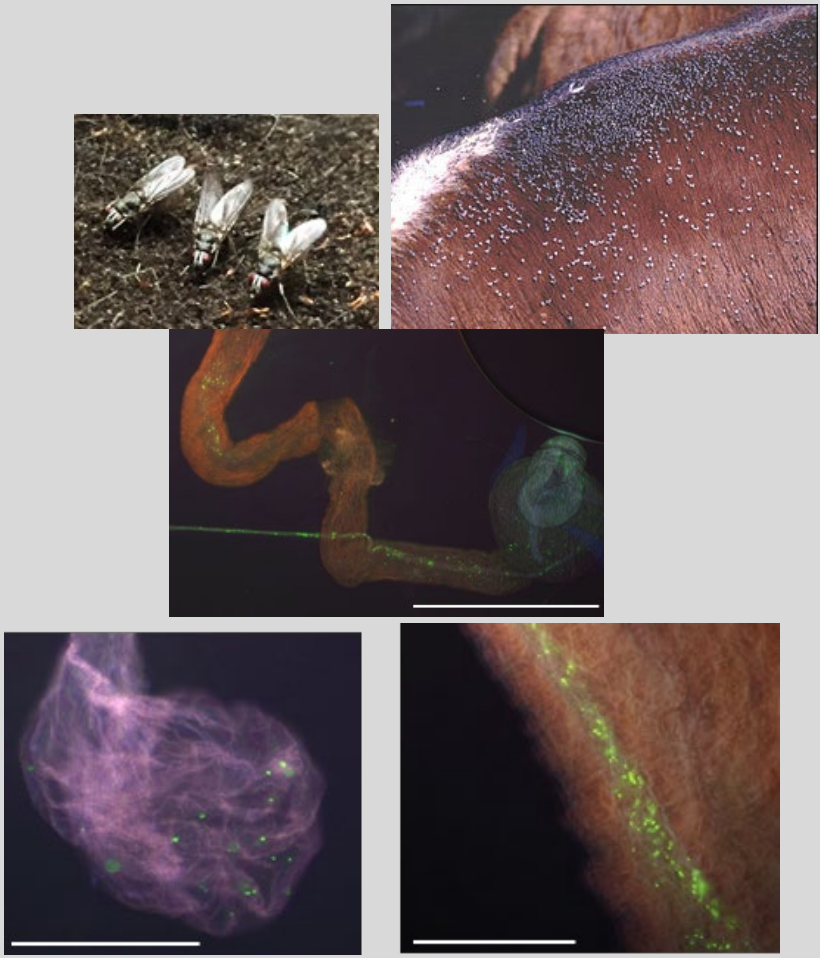
Impact: The efficacy of *P. protegens* as a topical application was surprising and appears to be due to bacterial production of fast-acting extracellular insect toxins. Unlike toxins produced by *Bacillus thuringiensis*, these toxins do not have to be ingested. The work opens new possibilities for development of *P. protegens* as a management tool for adult and larval filth flies, both alone and in combination with *B. bassiana*.

Horn Flies are Mechanical Vectors of *Salmonella* (2D)

Statement of Problem: Horn flies are of economic significance to cattle producers worldwide due to reductions in weight gain, hide damage, and annoyance to cattle. Horn flies rapidly develop resistance to synthetic insecticides, and there is a significant need for development of novel fly control technologies.

Accomplishment: A role for biting flies in the mechanical transmission of pathogenic bacteria to cattle was highlighted using the horn fly as a model. Studies demonstrated that a heavy infestation of horn flies carrying *Salmonella* can result in transdermal transmission of the bacteria to cattle, resulting in harborage of the bacteria in peripheral lymph nodes.

Impact: Understanding the role that biting flies play in the spread of microbial pathogens within livestock production systems will inform producers about the importance of fly management practices. Increased knowledge of bacterial fly relationships will enable improved food safety and animal health practices.



Salmonella acquisition by horn flies subsequent to tactile exposure and grooming

Sequencing and Assembling the Genome of the Horn Fly (2D)

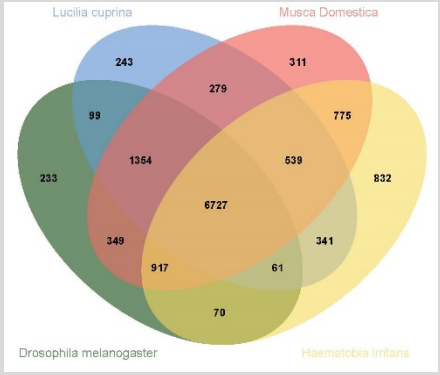
Statement of Problem: The horn fly has developed resistance to several insecticides and novel control technologies are needed by cattle producers. The genome is like a master template that guides the fly's development, metabolism, and responses to environmental perturbations, determining the fly's success within its ecosystem.

Accomplishment: Collaborating with NCGR and TAMU sequencing and bioinformatics experts, ARS scientists sequenced and assembled the genome of the horn fly and identified the full complement of genes.

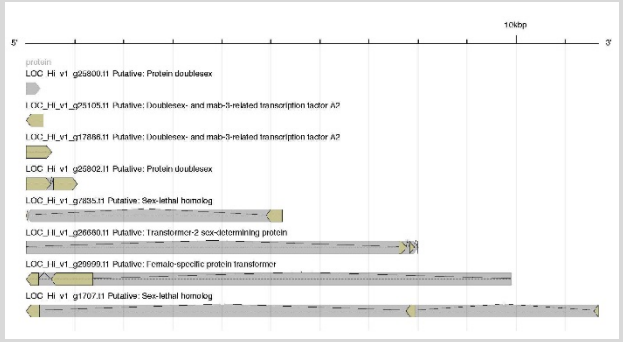
Impact: The data has been published and released to the scientific community. Knowing these gene sequences will facilitate development of novel fly control strategies aimed at specific fly genes and proteins targeted by new pesticides or anti-fly vaccines.

Key Points:

Horn flies feeding on cow



Horn fly-specific genes



Sex determination pathway genes

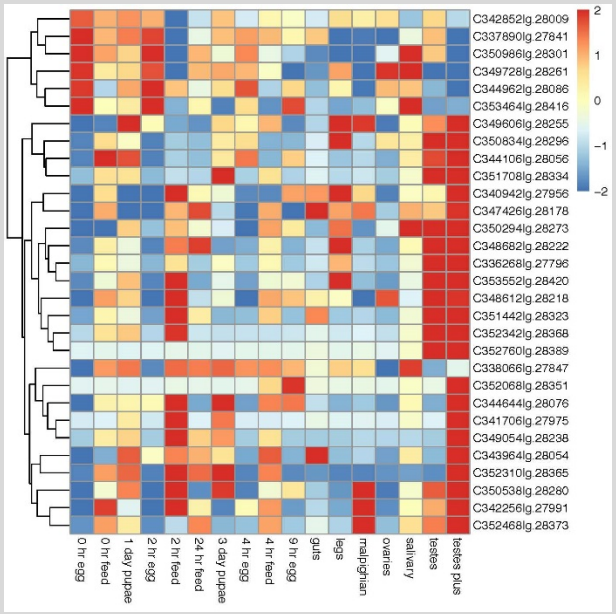
Tissue-Specific Transcriptomes of the Horn Fly (2D)

Statement of Problem: A comprehensive transcriptome for the horn fly was not available. Gene-based research on horn flies was hampered by the need to discover genes and transcripts by less efficient methods, one at a time.

Accomplishment: Collaborating with NCGR and TAMU sequencing and bioinformatics experts, ARS scientists sequenced, assembled, and annotated the transcriptomes of 17 different tissues and life stages of the horn fly, producing a transcriptome with full complement of fly genes.

Impact: The data has been published and released to the scientific community. Knowing these sequences and expression patterns has accelerated anti-horn fly vaccine antigen discovery.

Key Point:



Tissue-specific transcript expression patterns can now be visualized. This will assist studies aimed at:

- targeted gene silencing
- vaccine targets
- target tissues for new pesticide development

Production of Transgenic Male-Only Screwworm Flies (2E)

Statement of Problem: The New World screwworm fly, *Cochliomyia hominivorax*, was eradicated from the North American mainland by use of the sterile insect technique. Establishment of a barrier zone in Panama prevents fly reentry and establishment. The barrier is maintained by release of radiation sterilized male flies produced at Pacora, Panama. Currently both male and female flies are reared and released. Production of sterile flies for release is costly and produces substantial amounts of waste material of various types.

Accomplishment: A transgenic male-only strain was developed, laboratory evaluated, and is ready for field trials to evaluate performance compared to non-engineered strains.

Impact: Full production of transgenic male screwworm flies for sterilization and release will reduce waste and production costs by 50% because only male flies are reared in large numbers.

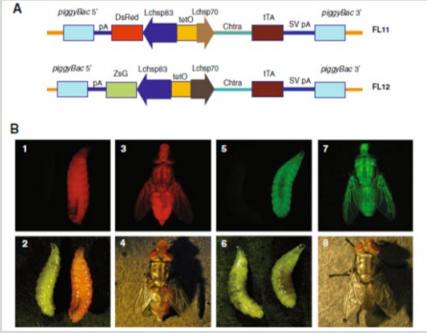
Concha *et al. BMC Biology* (2016) 14:72
DOI 10.1186/s12915-016-0296-8

BMC Biology

RESEARCH ARTICLE Open Access

A transgenic male-only strain of the New World screwworm for an improved control program using the sterile insect technique

Carolina Concha[1,2,3], Azhahianambi Palavesam[4,10], Felix D. Guerrero[4], Agustin Sagel[5], Fang Li[1], Jason A. Osborne[6], Yillian Hernandez[2], Trinidad Pardo[5], Gladys Quintero[5], Mario Vasquez[5], Gwen P. Keller[7], Pamela L. Phillips[5,8], John B. Welch[9], W. Owen McMillan[3], Steven R. Skoda[5,8] and Maxwell J. Scott[1*]



Entomologia Experimentalis et Applicata

DOI: 10.1111/eea.12607

SPECIAL ISSUE – STERILE INSECT TECHNIQUE

Review of research advances in the screwworm eradication program over the past 25 years

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Accepted: 4 May 2017

Reformulated Rearing Medium Leads to Improved Screwworm Production Efficiency (2E)

Statement of Problem: Production of the New World screwworm fly, *Cochliomyia hominivorax*, for use in eradication efforts and maintenance of the barrier zone in Panama is costly and produces substantial amounts of environmentally damaging waste material of various types. Refinements of rearing medium formulation to reduce use of high cost components and reduce generation of toxic or environmentally damaging waste would benefit the program.

Accomplishment: The gel formulation for rearing screwworm larvae was replaced with cellulose fiber. Increased production and release of toxic ammonia from the cellulose fiber-based medium was mitigated by addition of Yucca extract and potassium permanganate. Four volatile ovipositional attractants were identified that are expected to increase the number of eggs successfully produced for inoculation of screwworm larval production medium and will reduce the production cost and number of fertile females needed.

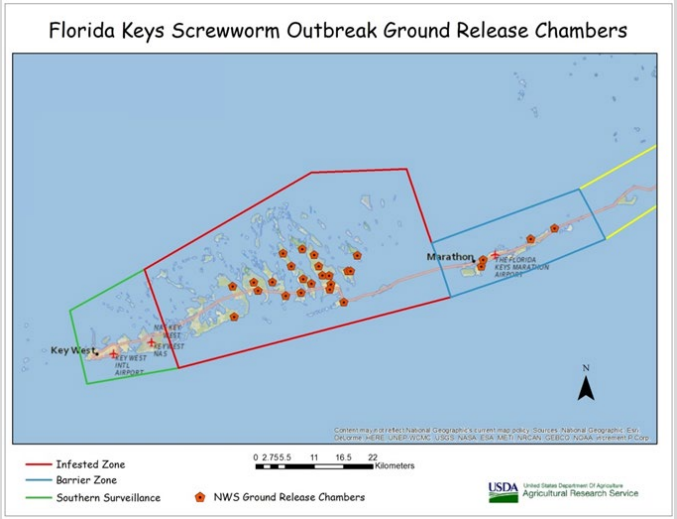
Impact: Reformulation of rearing medium used in screwworm production resulted in reduced screwworm production costs and production of more environmentally friendly waste material.

Eradicating a Screwworm Outbreak in Florida Hinged upon Contributions from ARS Scientists(2E)

Statement of Problem: On 30 September 2016 an outbreak of the New World screwworm, *Cochliomyia hominivorax,* was confirmed in the Florida Keys. ARS scientists responded to the outbreak following existing emergency response plans.

Accomplishment: Larval samples were collected from infested animals for DNA analyses, to determine mating compatibility with the factory reared strain, and to develop a Florida outbreak line. Habitat analysis was performed to identify locations for placement of ground release chambers (GRC) for dispersal of sterile flies. The first ground release was on 11 October 2016 and the last release was on 25 April 2017. A total of 30 GRC locations were established in the Florida Keys and 4 locations on the Mainland of Florida with an estimated 188 million sterile flies released over a period of 24 weeks resulting in successful eradication.

Impact: Successful eradication of screwworm flies from the Florida Keys in a relatively short amount of time prevented further spread of the screwworm infestation within the Florida, U.S. mainland. This was the first screwworm outbreak in the U.S. to be eradicated using only GRC's placed according to habitat analysis for dispersal of sterile flies, resulting in a significant cost savings and rapid eradication.

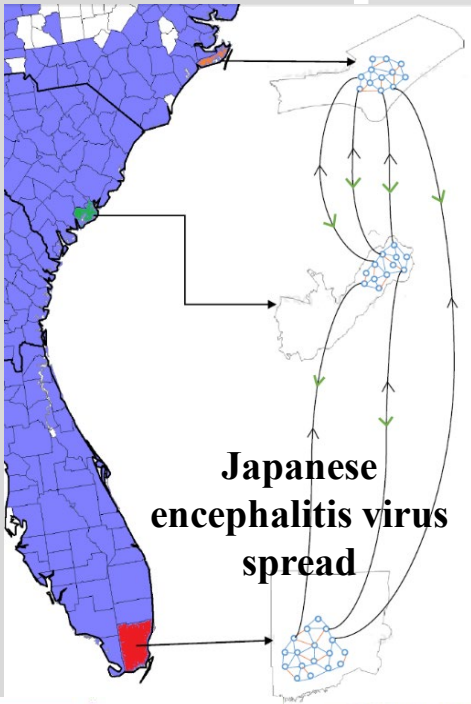


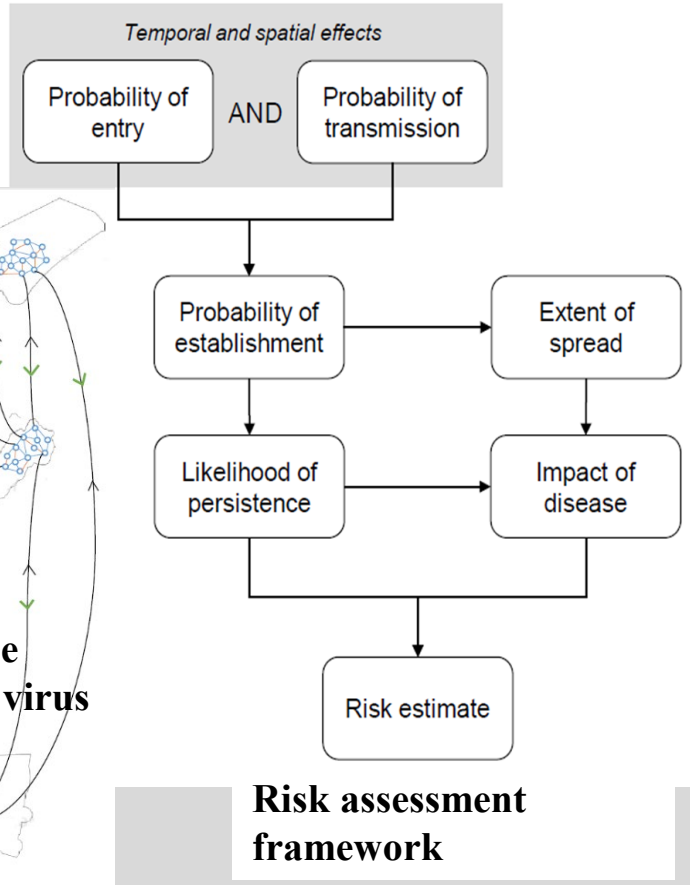
Genetic, Mathematical, and Epidemiological Mosquito Modeling (2F)

Statement of Problem: Livestock and poultry are heavily exposed to mosquitoes and therefore are a high risk for potential introduction of damaging invasive viruses such as Rift Valley fever and Japanese encephalitis.

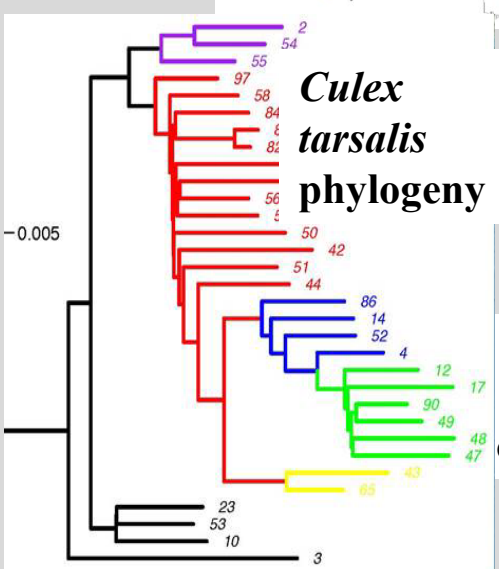
Accomplishment: ARS and University collaborators generated risk assessment and geographic expansion models based the genetic relationships between mosquito populations, animal hosts/reservoirs, and environmental/ weather characteristics.

Impact: The models are used by federal and state emergency planning agencies because they identify high risk areas for pathogen introduction and spread, key environmental factors, mitigation methods, and the traits of key mosquito species.

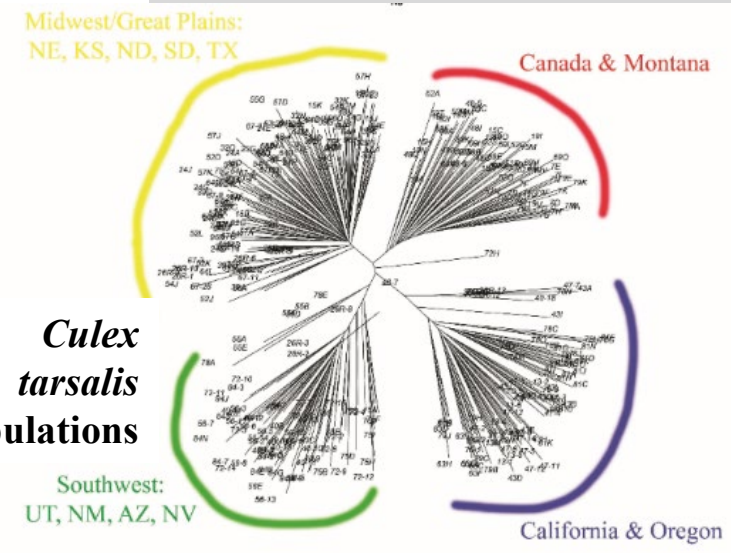




Risk assessment framework



***Culex tarsalis* phylogeny**



***Culex tarsalis* opulations**

Citizen Science Based Mosquito Monitoring Network (2F)

Statement of Problem: Single season continental mosquito collections require extensive collaboration to have effective geographic coverage and reduced travel/collection costs.

Accomplishment: The Invasive Mosquito Project puts the “public” back in public health by defining the roles of individuals in mosquito monitoring and control, which helps protect both humans and animals from mosquitoes and the pathogens they transmit.

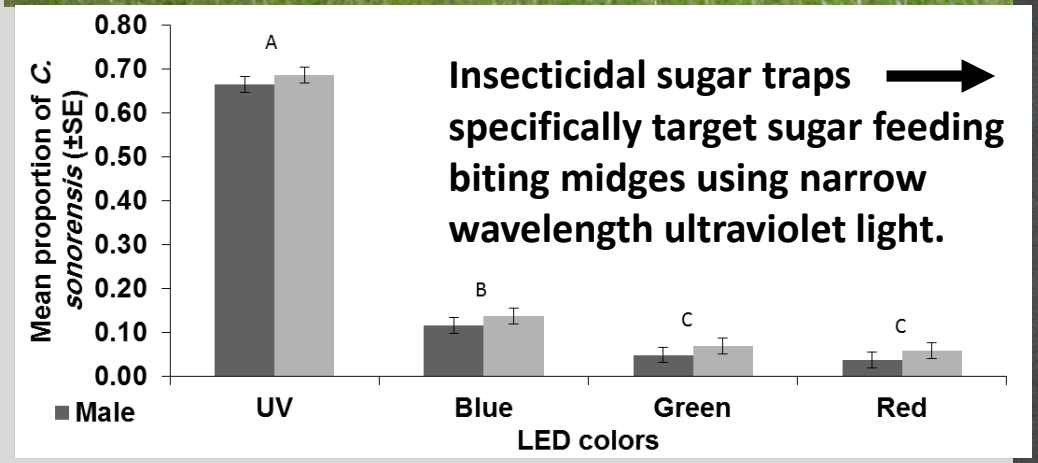
Impact: The citizen science collected mosquitoes increased stakeholder/ community engagement at the local farm or mosquito district level by providing a unified national education plan. The collected mosquitoes were used for genetic, mathematical, and epidemiological modeling studies and the farm level network nodes were used to introduce the Integrated Pest Management plan for small farms.

Mosquito oviposition traps

Integrated Pest Management Plan for Small Farms (2G)

Statement of Problem: Annually the captive cervid industry has a 7.9 billion dollar economic impact, directly generates 2 billion dollars, and employs 56,320 mainly rural people. Biting midge transmitted pathogens are the greatest killer of white tailed deer and captive cervids in the United States.

Accomplishment: ARS and industry stakeholders created the first customizable disease vector management plan for biting midges. The plan consists of novel tools such as treated barriers, aerial sprays, insecticidal sugar traps, larval habitat treatments, and deer quarantines.



Impact: The plan and tools have been implemented nationally using the farms network and have resulted in a significant reduction in captive cervid deaths from biting midge transmitted pathogens.

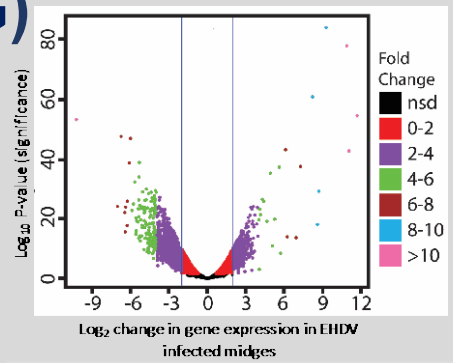
Developing Molecular Genetic and "-omic" Resources for Biting Midges (2G)

Problem: *Culicoides* midges transmit hemorrhagic disease orbiviruses to livestock (cattle, sheep) and deer, causing morbidity and mortality, impacting production and resulting in trade restrictions. While molecular genetic and big data "-omic" resources such as genomes, transcriptomes and proteomes, are available for other important disease vectors like mosquitoes and ticks, they have been lacking for biting midges.

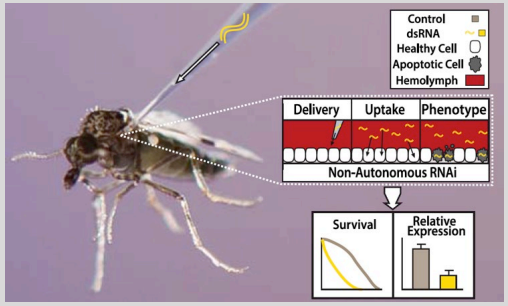
Accomplishments: ARS and university collaborators developed several resources for *Culicoides sonorensis* midges including: (1) a *de novo* transcriptome and related RNAseq analyses that cataloged gene products involved in key biological processes such as vector-virus interactions (e.g., EHDV), egg production, blood feeding and digestion, and innate immune responses to gut microbiome, (2) a salivary proteome, using a novel midge salivary protein collection method with 45 secreted salivary proteins identified by mass spectroscopy and revealing a myriad functions in facilitating blood feeding and modulating mammalian immune responses to midge feeding, (3) RNA-interference system developed to be used as a reverse-genetics tool to understand gene function as it relates to vector competence for orbiviruses.

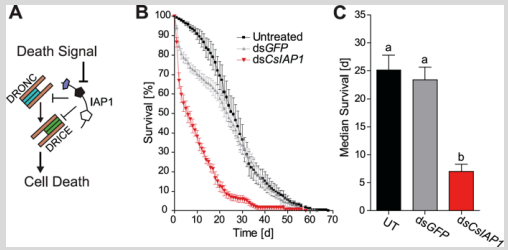
Impact: These publicly-available tools have provided much-needed resources for identifying the molecular components that underlie important processes in the midge (reproduction, vector competence, vector-host interactions, transmission). Such studies can reveal novel mitigation opportunities and key transmission-blocking targets.

Female biting midge, *Culicoides sonorensis*



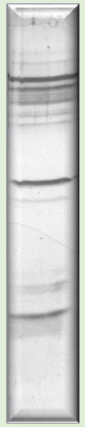
RNAseq analysis revealed genes that were differentially-expressed between control and EHDV-fed midges. Each dot represents a gene whose expression was either down (left of 0) or up (right of 0) due to virus infection. Vertical axis is statistical significance of that difference.





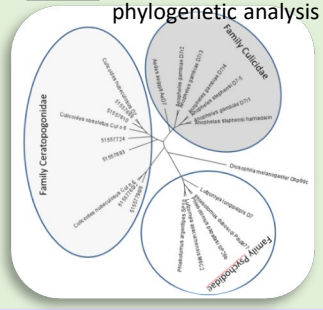
RNA-interference was developed and tested using injected dsRNA. Injection of dsRNA to a target gene (A. IAP1, which controls cell death) resulted in a an increased mortality phenotype (B) and measurable downregulation of that gene (C) in female midges.

C. sonorensis salivary proteome



Salivary protein collection

D7 Superfamily phylogenetic analysis



Questions on Component 2?

Component 3: Fire ants and other invasive pests

Problem Statement 3A: Invasive fire ants

Problem Statement 3B: Invasive crazy ants

Problem Statement 3C: Other invasive pest ants and climate change

Pest Ant Basic Research Leads to Practical Control Methods (3A)

Problem: Fire Ants cause over 6 billion dollars in annual costs for control and damage repair for many economic sectors, including Agriculture and Livestock. Improved and new methods of pest ant control are needed, especially biologically-based methods that are species specific.

Results & Impact.
PART A. Research to characterize fire ant PBAN and its receptor led to fire ant and moth control possibilities

- Basic fire ant research resulted in the characterization of the Pheromone Biosynthesis Activation Neuropeptide (PBAN) ligand and its receptor (PBAN-R).
- The PBAN gene was expressed in all fire ant life forms, suggesting multiple functions.
- RNAi suppression of gene PBAN expression in larvae by feeding led to larval mortality.
- This model (PBAN suppression) has been extended to some Lepidopteran pests where larval growth is inhibited and few larvae survive to adulthood.
- Three patents have issued and others are in patent office review.
- There is commercial interest.

Pest Ant Basic Research Leads to Practical Control Methods (3A)

Part B. Bioactive peptides have insecticide activity

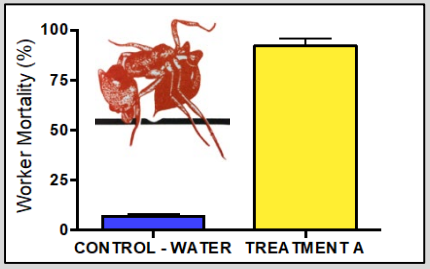
- A unique method for screening hundreds of thousands of small peptides for strong binding activity against a cell line expressed PBAN receptor, yielded several unique sequences.
- Some of these peptides when injected into worker ants caused mortality.
- Some of these peptides, in a sucrose solution, showed mortality effects when fed to workers. There is commercial interest in this technology
  - (Choi, M.-Y. and Vander Meer, R.K., 2017. Bioactive Peptides having Insecticide Activity.
    U.S. Patent No. 9,771,393 B2; another Patent Application is pending).

EXAMPLE PEPTIDES

MKFIQALSIS
QESLADDGVG
TFIDQGSPTQ
YNIVEQLAYN
VVSSDGNGNC

Part C. Males transfer unique compounds to females during mating where they are then hydrolyzed to a bioactive neurohormone

- The neurohormone triggers numerous physiological events in the newly mated queens, such as ovariole development and pheromone production. The neurohormone elicited the same physiological changes when injected or fed to mature female sexuals.
- When fed to worker fire ants, the neurohormone caused mortality. The compound has
  delayed activity over a range of concentrations, which support its use as a fire ant bait toxicant (see graph on right).
- This discovery is protected by two patent applications and was funded in part through a CRADA and SBIR Phase I grant. Development and commercialization will be facilitated with a recently funded Phase II SBIR Grant.



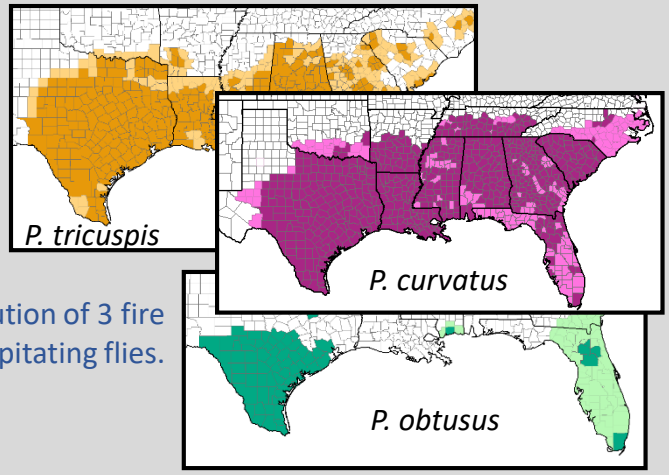
Fire Ant Biocontrol: Decapitating Flies (3A)

Problem

• Fire ants are well established in the southern U.S., infesting over
367 million acres encompassing all types of habitats.

• Fire ants are 5-10 times more abundant in the U.S. than their native home land because of escape from natural enemies.

Fire ant decapitating fly attacking fire ant.



Distribution of 3 fire ant decapitating flies.

Accomplishments

- Monitored long-term changes in imported fire ant populations at 192 sites in Florida and Georgia.
- Developed rearing procedures, obtained permits and released additional fly species for a total of 6, with the assistance of APHIS and other cooperators at dozens of sites in the U.S.
- Released *P. nocens* at 2 sites in Gainesville, FL
- Monitored establishment and expansion of two populations of *P. cultellatus* in Florida.

Impacts

• Decapitating Flies are parasitizing fire ants throughout the U.S. over a variety of habitats (see maps).

• Multiple surveys show an association between the reduction in fire ant populations and establishment of the decapitating flies.

Fire Ant Biocontrol: Viruses (3A)

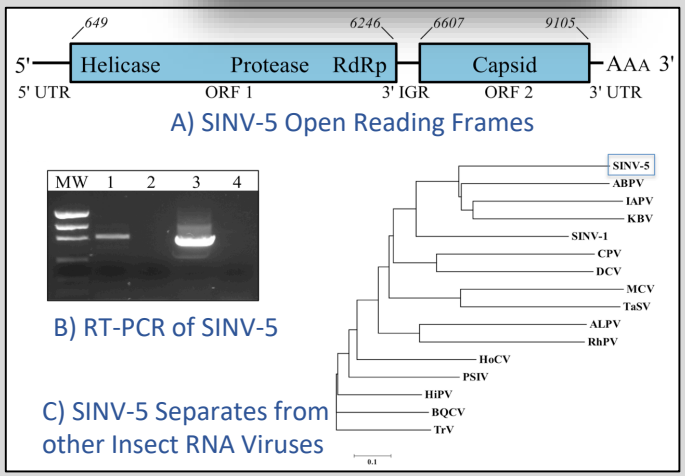
Problem

- Red imported fire ants (RIFA) in the U.S. are nearly devoid of natural enemies.
- Discovery and release of natural enemies (e.g. viruses) into the U.S. population of RIFA can provide sustainable control of this invasive pest.

Results and Impact

- Gene library preparation, sequencing and analysis of colonies collected from the native range was completed in an effort to discover new biocontrol agents for RIFA.
- Resulted in the discovery of at least 3 new viruses specific to RIFA, which may be important natural control agents for the ant.
- One virus appears to be only found in South America (SINV-5).
- The virulent Solenopsis Invicta Virus-3 (SINV-3) was released and is spreading in the Coachella Valley, CA

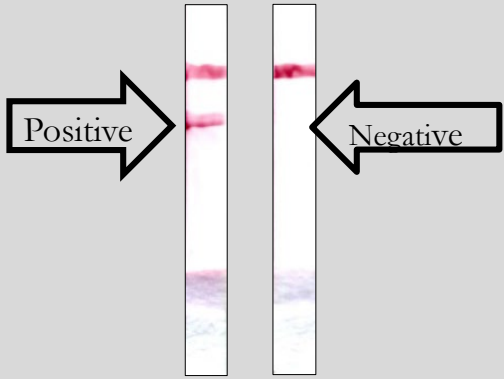
Twenty-Five Fire Ant Collection Sites



Improving IPM of Fire Ants with Detection (3A)

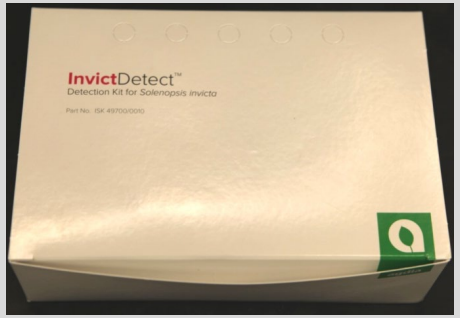
Problem

- Visual identification of imported fire ants to species is difficult, requiring considerable expertise in ant taxonomy and is prone to error.
- APHIS quarantine of imported fire ants was established to prevent their human-assisted spread. A rapid method of taxonomic identification of red imported fire ants was needed to facilitate detection at quarantine boundaries and other points of potential introduction.



Results and Impact

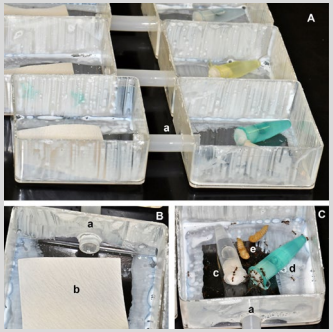
- Developed a rapid, field-portable lateral flow immunoassay capable of identifying red imported fire ants from all other ant species, including native fire ants.
- The method utilizes custom-made monoclonal antibody pairs in a lateral flow format to detect Solenopsis invicta venom 2—a unique venom protein of *Solenopsis* ant species.
- This technology was transferred to private industry via a Biological Material License Agreement to Agdia, Inc. and commercial production and availability of InvictDetect®. Kits are available for purchase from Agdia, Inc.
- In addition to APHIS in the U.S., biosecurity agencies in Australia, Korea, and Japan are intending to purchase and use the kits for detection of fire ants.



Entomopathogenic Fungi for Fire Ant Control (3A)

Statement of Problem: New effective and environmentally friendly products to control invasive ants are needed. Use of entomopathogenic fungi have proven ineffective to produce mortality to these ants due to ants ability to combat pathogens.

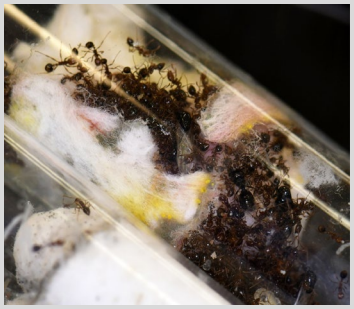
Accomplishments: Indirect exposure to *Beauveria bassiana* NI8 sprayed to filter paper squares caused significantly higher mortality to ant workers than other two fungi tested and control. Additionally, defatted *Camelina sativa* meal by-product (with high content of natural toxins) was readily consumed by ant workers in a choice test producing detrimental effects to the ant colony; but no detrimental effect to the fungal spores.



A) Experimental arenas(a). B) One side contained a square of filter paper either blank or sprayed with fungal spores (b). C) The second side contained 50 fire ant workers provided with water (c), a sugar solution (d), and two *T. molitor* pupae (e)

Cadaver of a fire ant worker showing *B. bassiana* growth in PDA plate after incubation

Fire ant colony in choice test exposed to *C. sativa*

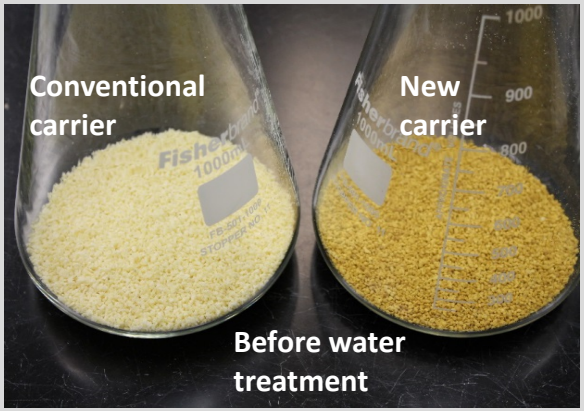


Presence of fungus and dead fire ant brood in tube nest after a month of exposure to *Camelina* residues

Impact: The development of a dual indirect bioassay system to test fungal spores allowed to determined mortality caused by each strain by post mortem isolation. The fact that ant workers were infected by indirect contact to the spores and not by direct spraying is significant because it demonstrates that *B. bassiana* NI8 strain spores can be used to implement a microbial bait. Further, the addition of *C. sativa* meal by-product seems to be acting as a synergist which will enhance the efficacy of such bait (Research in progress).

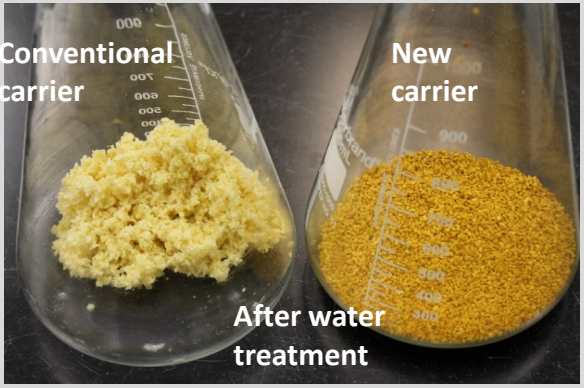
New Fire Ant Bait Carriers (3A)

Statement of Problem: Baits are commonly used in the imported fire ant management. Currently, most fire ant bait formulations in the market use pre-gelled defatted corn grit as a carrier. Since corn grit is easily dissolved in water, corn grit based fire ant bait can only be used when the ground and grass are dry and rain is not expected. This has been a significantly limiting factor on the use of bait products in fire ant management.



Accomplishment: Novel bait carriers were developed, independently or collaboratively with CRADA partners, Those carriers significantly improved water resistance of fire ant baits and increased acceptance by fire ants as well.

Impact: This work led to a CRADA with McLaughlin Gormley King Company (MGK) for developing new insect control products and a CRADA with Cargill Inc. for developing new water resistant corn grits. MGK has used the new bait carrier in their product development. The work with Cargill Inc. resulted in a new water resistant grit that can be used for formulating both oil and water based ant baits. These bait carriers can enhance the bait efficacy by improving bait attractiveness and stability and increasing the effective coverage time of the bait.



Comparative Study on Red and Black Imported Fire Ants (3A)

Statement of Problem: The red imported fire ant (RIFA) is a very successful invasive species. The black imported fire ant (BIFA) is a closely related invasive species to RIFA, but with much less success. Although BIFA was introduced and established more than one decade earlier than RIFA in the United States, the latter has gradually displaced the BIFA throughout most of their distribution area. Why this happened is a frequently asked question in fire ant research. Comparative study on these two species may help us identify species attributes that contribute to the invasion success of RIFA and predict its pattern of future spread.

Accomplishment : We discovered that RIFA has significantly higher tolerance to heat, desiccation and toxic stress than BIFA.

Impact : Difference in stress tolerance may contribute to the displacement of the BIFA by RIFA. The same dose is currently recommended in the chemical control for these two species. Since BIFA are significantly less tolerant to insecticides than RIFA, less insecticide than the recommended dose might be needed for an effective control of BIFA.

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Esterase in Imported Fire Ants, *Solenopsis invicta* and *S. richteri* (Hymenoptera: Formicidae): Activity, Kinetics and Variation

J. Chen, T. Rashid & G. Feng

Affiliations | Contributions | Corresponding author

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RESEARCH ARTICLE

A Comparative Study between *Solenopsis invicta* and *Solenopsis richteri* on Tolerance to Heat and Desiccation Stresses

Jian Chen, Tahir Rashid, Guolei Feng

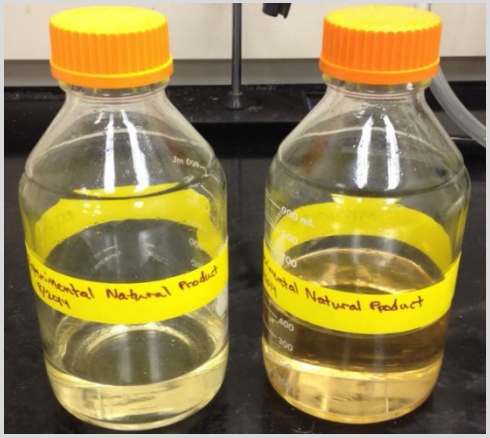
Published: June 10, 2014 • DOI: 10.1371/journal.pone.0096842

New Fire Ant Mound Treatment Formulations (3A)

Statement of Problem: Mound treatment with contact insecticide is a common practice in fire ant control. Numerous synthetic contact insecticides have been registered for fire ant control. Due to the ever increasing concern to the negative impact of synthetic insecticides on human health and environment, the use of more toxicologically and environmentally benign chemicals is highly desirable. Chemicals from natural products are believed to be more desirable insecticides than conventional synthetic insecticides, due to their rapid environmental biodegradable property and lower toxicity to natural enemies, humans and other mammals. However only few fire ant control products in the current market are based on naturally occurring compounds.

Accomplishment: We identified effective fire ant toxins from plant secondary metabolites and defensive chemicals of other ants, such as methyl ketone from the defensive secretion of tawny crazy ants and benzoate analogs from plants. Based on those compounds, several mound treatment formulations were developed.

Impact: One compound that shows high toxicity against fire ants has neither known OSHA hazards nor aquatic toxicity and one structurally related compound is even not considered as a hazardous substance, indicating a great potential for their application in fire ant management.

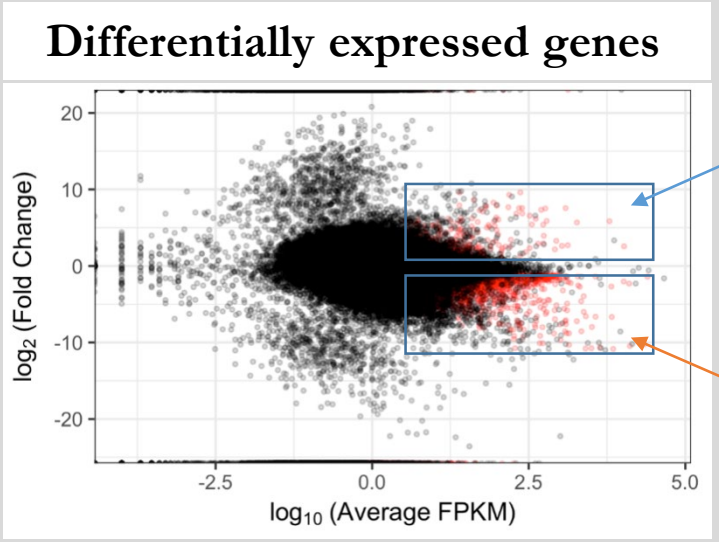


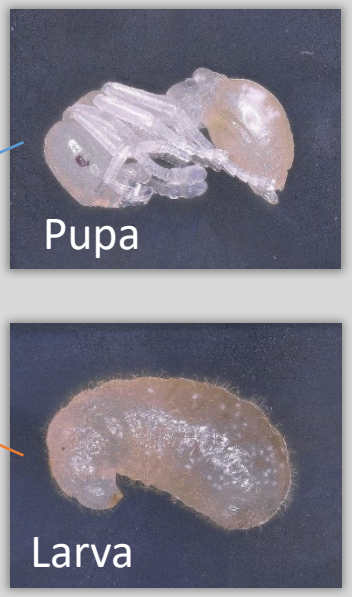
Formulations based on naturally occurring compounds for fire ant mound treatment

Enabling Genetic-Based Pest Control Development for Invasive Ants (3A)

Statement of Problem: New effective and environmentally friendly products to control invasive ants are needed. Gene-based products hold great promise because they can kill only the target species. While the genome of the Red Imported Fire Ant (RIFA) is available, selecting targets for gene interruption is a major challenge.

Accomplishments: Using next generation sequencing and bioinformatic analyses of expressed genes of larvae and pupae from three independent colonies of ants, ARS scientists identified hundreds of genes involved in vital physiological pathways. A new variety of positive strand RNA virus ($SINV2_{MS}$) was also identified.





Impact: Genes identified in this study will be used to test gene knockdown using RNA interference (RNAi) and other molecular methods. *All data sets have been deposited to the National Center for Biotechnology Information (NCBI) for further analysis and genetic pest control testing (BioProject number PRJNA393960).* The new variety of virus has great value as a transmission tool for genetic pest control constructs based on advancing gene interference technology such as RNA interference (RNAi).

Controls for the Invasive, Tawny Crazy Ant (3B)

Problem

- The tawny crazy ant, *Nylanderia fulva*, is an invasive ant from S. America that develops huge populations.
- Kills crops, reduces biodiversity, and infiltrates buildings; → decreases value of infested lands.
- Widely distributed in Florida and Texas; spreading among other Gulf Coast states.
- Available controls are temporary and inadequate; leads to excessive pesticide applications.

Results and Impact

- Discovered pathogens for potential biocontrol:
  - Nylanderia fulva virus -1 (patented)
  - Microsporidia *Myrmecomorba nylanderiae* (with Univ. Texas)
  - ***Biological control is the only self-sustaining method to suppress established invasive ants.***
- Obtained visually perceptible reductions in field populations of TCA with liquid ant bait.
  - ***Bait can reduce pesticide (AI) applied 99.4%.***

"This land here is toxic, Georges said. I've had these ants for almost two years and I have tried every pesticide known to man. Nothing kills em."

Photo (L): handfuls of dead TCA

New Sample Preparation Method for Analyzing Insect Volatiles (3C,3A,3B)

Statement of Problem: Classical sample preparation methods for insect pheromone analysis involve solvent extraction of insects, which often requires a great number of tested insects and huge volume of solvent. In the past two decades, one technique has been getting more and more popular in insect pheromone analysis is the headspace solid-phase microextraction (HS-SPME). In HS-SPME, the overall amount of volatiles that can be collected for chemical analysis is proportioned to the concentration of volatiles in head space that is largely determined by amount of volatiles insects indeed release into the head space. In many cases, active insect volatiles, such as insect pheromones, are produced in very low quantities.

Accomplishment : An innovative sample preparation method for HS-SPME was developed by an ARS scientist, which significantly improves the detection and analysis of insect volatiles. In this method, a freeze-thaw process was applied to insect samples before the conventional SPME extraction.

Impact : The application of this innovation may significantly facilitate the identification of insect semiochemicals, such as insect pheromones.

analytical chemistry

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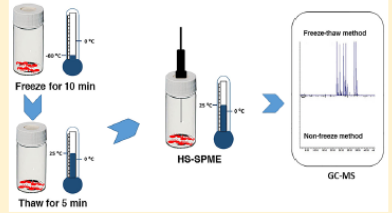
Freeze–Thaw Sample Preparation Method Improves Detection of Volatile Compounds in Insects Using Headspace Solid-Phase Microextraction

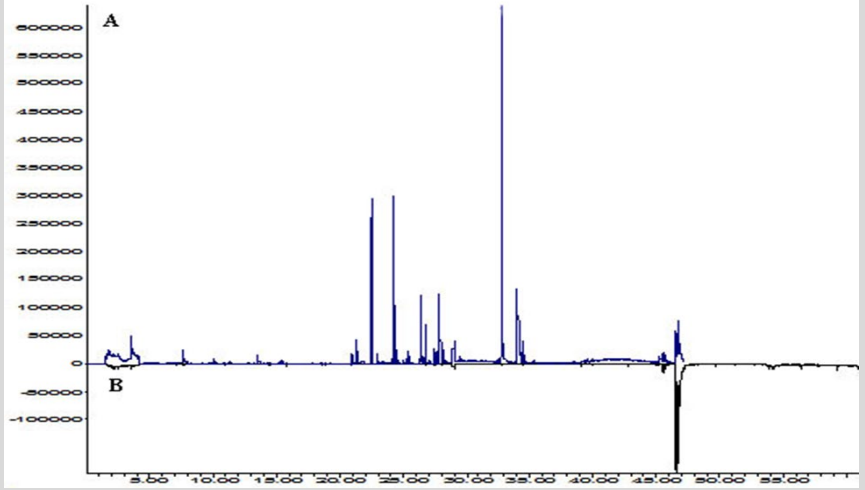
Jian Chen*

National Biological Control Laboratory, USDA-ARS, 59 Lee Road, Stoneville, Mississippi 38776, United States

Supporting Information

ABSTRACT: Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) is commonly used in analyzing insect volatiles. To improve the detection of volatiles in insects, a freeze–thaw method was applied to insect samples before the HS-SPME–GC–MS analysis. Insect samples were first frozen at −80 °C for 10 min and then thawed at 25 °C for 5 min before SPME extraction was performed. The freeze–thaw method clearly improved the detection of volatile compounds for all six tested insect species, including red imported fire ants, *Solenopsis invicta* Buren, black imported fire ants, *Solenopsis richteri* Forel, little black ants, *Monomorium minimum* (Buckley), pharaoh ants, *Monomorium pharaonis* (Linnaeus), eastern subterranean termites, *Reticulitermes flavipes* (Kollar), and spotted lady beetles, *Coleomegilla maculate* De Geer. This method helped identify various volatile compounds in the tested insects which have never been reported previously. This improved method may facilitate the identification of insect derived volatiles such as insect semiochemicals.





Overlay of GC–MS chromatograms comparing profiles of volatiles in *Monomorium minimum* workers between two sample preparation methods. A: freeze-thaw method, SPME 2h. B: conventional nonfreeze method, SPME 2h.

Questions on Component 3?

Panel Discussion