The USDA
Mosquito and Fly Research Unit
in Gainesville, Florida

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Mosquito and Fly Research Unit
Center for Medical, Agricultural, and Veterinary Entomology
Agricultural Research Service
USDA
Gainesville, Florida
The last 77 years

- 1932- USDA Division of Insects Affecting Man and Animals established an entomological research laboratory in Orlando → mosquitoes (including malaria vectors under conditions “simulating those of South Pacific jungles”) and other insects affecting man and animals.
- 1953- Agricultural Research Service (ARS) created
- 1961- Laboratory moved to University of Florida campus in Gainesville, Florida
- 1988- “Mosquito and Fly Research Unit” created
- 1996- “Center for Medical, Agricultural and Veterinary Entomology (CMAVE)” created
Summary of past accomplishments

1950s- Developed new method for **residual control of Anopheles**

1960s- Selected the broad-spectrum repellent **DEET** because of its effectiveness in protecting deployed troops from arthropod-borne diseases
  - Evaluated **over 30,000 compounds**

1960s and 1970s- Developed **mass-rearing technology for Anopheles**
  - Developed and conducted international assessment of the feasibility of the **sterile insect technique (SIT)** for mosquito control
  - Developed **ultra-low volume (ULV) pesticide spray systems** that provided area-wide protection of troops
Summary of past accomplishments II.

- Searched for and conducted biological studies and assessed a variety of biocontrol approaches, including microsporidia and viruses; worked extensively with *Toxorhynchites* mosquitoes.

1970’s-1980s  To reduce reliance on hard pesticides and introduce environmentally safer compounds, ARS made major contributions to the development and registration of methoprene, *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus*.

- Developed permethrin-impregnated tents and uniforms for personal protection of troops.
Summary of past accomplishments III.

1980s and 1990s - Developed innovative models of vector population dynamics
- With industry and the World Health Organization, assessed almost all of the public health insecticides entering the market place in the last half of the 20th century; as part of the ARS effort to reduce usage of hard pesticides and introduce environmentally safer compounds.
- Elucidated the taxonomy of cryptic species of the Anopheles quadrimaculatus complex
- Extensively characterized the molecular biology of vector mosquito species
Research team

- **Specialists** in molecular biology, chemistry, toxicology, microbiology, virology, insect behavior, biology, entomology, vector ecology, botany, geographic information systems and engineering.
- **Staff** conduct research at the basic (chemical-molecular) level and later evaluate their results in laboratory bioassays and under semi-field conditions. Final studies are conducted at field sites in the U.S. and internationally, as necessary.
- Unit has produced over 2,500 publications
- A unique national and international resource
Current MFRU staff

Ken Linthicum
CMAVE Director

Gary Clark
Research Leader

Seth Britch
Entomologist

Sandra Allan
Lead Scientist
Sensory Ecology and Surveillance

Jerome Hogsette
Lead Scientist
Biology and Management Higher Diptera

Ulrich Bernier
Lead Scientist
Biology and Control Biting Flies

Lee Cohnstaedt
Entomologist

Chris Geden
Entomologist Flies

Don Barnard
Entomologist

Dan Kline
Entomologist

James Becnel
Entomologist

Maia Tsikolia
Chemist

Monique Coy
Molecular biologist

Fund by Department of Defense’s
Deployed War Fighter Protection Program (DWFP)
MFRU infrastructure

- **Insectary space**: 4,000 → 11,000 sq ft
  - Mosquito species: 9
  - Fly species: 4
  - More mosquitoes in the future
- **Specialized equipment**
  - 8 10’ x 20’ and 3 10 x 12’ environmental chambers
- **Quarantine facility** with 10 parasitoid species and sand flies (*Phlebotomous papatasi*)
- **Outdoor cages**
Mosquito and fly species at MFRU

- Aedes aegypti
- Ae. albopictus
- Anopheles albimanus
- An. quadrimaculatus
- Culex nigripalpus
- Cx. quinquefasciatus
- Cx. salinarius
- Cx. tarsalis
- Ochlerotatus taeniorhynchus
- Musca domestica (House fly)
  - Susceptible
  - Pyrethroid resistant
  - 2005 "wild type"
- Stomoxys calcitrans (Stable fly)
- Hydrotaea aenescens (Black dump fly) (Sand fly)
- Sarcophaga bullata (Flesh fly) Phlebotomous papatasi
Chemical instrument to detect volatile organic compounds

Olfactometers used to assess efficacy of novel volatile organic compounds
Screened cages (30’ x 60’ x 16-18’ tall) for semi-field studies

Used after laboratory studies – prior to field testing
Tent inside large cage
New, 7,000 sq ft insectary built with DWFP funds
Tent city used to study behavior and control of house flies
Mosquito attractants and repellents

Chemicals produced on the skin attract mosquitoes for feeding. Effective attractant blends have been developed from these compounds and can potentially enhance trap collection.

Some compounds repel mosquitoes while others “mask” the chemical cues used for host location. These inhibitors can be used in a “push-pull” type system to enhance trap catches and minimize attraction of mosquitoes to humans and livestock.
Relative efficacy of commercial mosquito traps
What has the USDA mosquito and fly research program accomplished recently?
Recent surveillance results: mosquitoes

- Found that traps with oak and pine infusions at ground level were most attractive for female *Aedes albopictus*.
- Observed that human landing rates at specific times can accurately predict CDC LT captures.
- Found that attractant blend, trap location and mosquito species affect the efficacy of CDC LTs in capturing floodwater mosquitoes.
- Variables (soil type and urbanization) correlate with vector activity and may be incorporated into a predictive GIS-based model leading to identification of ideal locations for placement of mosquito traps in a vector surveillance system.
Recent surveillance results: mosquitoes

- Developing GIS models of climate-population and habitat-population associations to predict temporal and spatial distribution and abundance of mosquitoes.
- Examined effects of different attractants on flight characteristics (flight initiation, duration of flight, directness and altitude of flight) of *Aedes* spp. in a wind tunnel; responses may help optimize attractant use/trap design.
Recent control results: mosquitoes

• Found that expression of 8 genes was higher in permethrin-treated *Aedes aegypti* than in acetone-treated *Ae. aegypti*. These genes can be targeted using newly developed technology to silence genes (RNA interference) for resistance management and toxicity.

• Evaluated toxicities of 33 carboxamides against female *Ae. aegypti* by topical application and determined LD50 and LD95 values.
Recent control results: mosquitoes

- Prallethrin increased speed and duration of mosquito flight -> deposition of more pesticide droplet and higher mortality.
- Developed a wind tunnel assay to compare the effects of sub-lethal exposure to permethrin and deltamethrin on sensory responses in three genera of mosquitoes to several host-attractant compounds. The behavioral impact on flight orientation to host odors after pesticide exposure indicated differences between species and reduced flight after exposure to sub-lethal doses.
Recent control results

• Found that barrier treatments of natural vegetation reduced mosquito numbers for up to a month.
• Treatment of U.S. military desert and woodland camouflage netting and tent systems reduced mosquito and sand fly numbers for up to 8 months.
• Evaluated bite protection afforded by new permethrin-treated US military uniforms.
• Developed a technique that allows evaluation of both toxicity and spatial repellency of a chemical to be evaluated under semi-field and field conditions-> may lead to more innovative vector control strategies.
Recent research results: flies

- Blue-black cloth stable fly targets impregnated with 0.1% lambda-cyhalothrin were formed into cylinders and reduced in size, making them more stable and more convenient for use without reducing efficacy.

- Stable flies captured at a study site were covered with pollen. The pollen was identified as being from willow trees, indicating the route the flies used to reach the study site.
Recent research results: flies

- Imidacloprid–treated visual targets were evaluated and determined to effectively reduce the numbers of house flies entering a calf barn.
- Studies demonstrated that when the amount of ultraviolet light released from traps placed indoors for house fly management was reduced, significantly fewer flies were captured and killed.
- Host attractant compounds in combination with carbon dioxide were found to significantly enhance collection of *Lutzomyia* sand flies in commercial traps fitted with either red or near-UV LED lights.
International involvement

- Organized key researchers and administrators from federal and state agencies and universities into the Rift Valley Fever (RVF) Working Group to protect the US against the possible arrival of RVF and other mosquito-borne viruses.
- Three inhibitors formulated in nanoclays were tested and found to suppress host-seeking behavior in *Phlebotomus papatasi* sand flies in Egypt.
- Had a significant role in development of new WHO guidelines on efficacy testing of (1) mosquito repellants for human skin; (2) insecticides for indoor and outdoor use ground-applied space spray applications, and (3) household insecticide products.
Area-Wide Project for Management of the Asian Tiger Mosquito
Participating groups

- Agricultural Research Service, USDA
- New Jersey Agricultural Research Station
- Brandeis University (Massachusetts)
- Mercer County (NJ) Mosquito Control Program
- Monmouth County (NJ) Mosquito Extermination Commission
The target: Asian Tiger Mosquito 
(*Aedes albopictus*)

Photograph: Courtesy Susan Ellis
Agricultural Research Service (ARS) Area-Wide Pest Management Initiative

Initiative implemented in 1995 to demonstrate the positive impacts and advantages of adopting a large-scale approach to end users affected by pests.

Expected outcomes:

1. increased profits or health benefits
2. reduced worker risks from chemical pesticides
3. enhanced environment
4. demonstrated superiority of a strategy implemented over a wide geographic area where the pest is present
Area-Wide Integrated Pest Management (IPM) Approach

• Centered on management of pests where existing pest control technologies (e.g., chemical, physical, biological and cultural) are most effective when used over a multi-state or multi-regional area.

• Focuses on reducing and maintaining pest populations at an acceptable (low) density.

• This is the first ARS area-wide project to target a mosquito species.
Relevancy to ARS

- National Program (NP) 104 “Veterinary, Medical and Urban Entomology” led by Daniel Strickman
- The goal of NP-104 is to perform research that contributes to efficient and safe protection of humans and animals from damage caused by arthropods.
- The Center for Medical, Agricultural and Veterinary Entomology (CMAVE) in Florida has been involved with mosquito research for many decades.
Distribution of *Aedes albopictus* in 2005 and predicted suitable range*

Aedes albopictus: The current situation

• Detected in the U.S. in the mid-1980s; eggs arrived in multiple introductions via international commerce in used truck and automobile tires

• Rapidly spread to many states via domestic commerce of used tires; now found in >30 states

• Naturally infected with several arboviruses (e.g., West Nile [USA], dengue and chikungunya) and is a competent vector in the laboratory for several other arboviruses (e.g., Eastern equine encephalitis [USA] and LaCrosse [USA] and Rift Valley fever, Japanese encephalitis, Ross River, and yellow fever)
Overall objectives of the project

- Demonstrate an effective strategy for area-wide control of Aedes albopictus
- Demonstrate the public health importance and socio-economic benefits of area-wide mosquito control
- Transfer the effective technology and strategy to end users
Specific objectives of this project

- Reduce adult populations of *Aedes albopictus* by 95% (as measured by B-G traps) in areas that are at least 4 km² in size
- Evaluate cost-benefit operational effectiveness of each surveillance and control method (cost/nuisance/health)
- Establish strategies for maintenance of area-wide reduction of *Ae. albopictus* populations by local control programs
- Develop a manual for mosquito abatement districts
Unique aspect of this project

- Economists at Brandeis University will calculate socio-economic cost/benefits of mosquito control.
- Brandeis team has international experience in studying and measuring costs and benefits of vector-borne diseases (e.g., dengue).
- They will conduct key interviews and surveys to:
  - measure public perception of nuisance and personal costs.
  - measure costs and economic feasibility at the state/county level.
  - measure social and health (humans/livestock) costs.
Projected outcome

Describe and demonstrate economical, sustainable and reliable methods that combine existing technologies to eliminate the threat of *Aedes albopictus* as a pest and disease vector in any U.S. community with an integrated mosquito management program.
Potential benefits to agriculture

- **Reduced threat to livestock** of domestic (EEE) and certain invasive (WNV and Rift Valley fever) mosquito-borne viral pathogens
- **Reduced threat to humans** of these and other arboviruses
- **Improved quality of life** for agricultural workers active in areas where these viruses are transmitted
- **Establishment of methods** that are applicable to area-wide management of other mosquito species
DoD Deployed War Fighter Protection (DWFP) Research Program

Novel insecticide chemistries or formulations

Personal protection

Application technology
MFRU and DWFP

• Field studies in the U.S. and Kenya/Lab studies with infected mosquitoes- Ken Linthicum
• Search for new toxicants and methods for control- James Becnel
• New insecticides and repellents for mosquitoes and sand flies- Uli Bernier
• Evaluation of house fly traps under desert conditions- Jerry Hogsette
MFRU and DWFP cont.

• Evaluation of spatial repellents under semi-field and field conditions- Dan Kline
• Sand fly and mosquito behavior responses to DUET and treated surfaces- Sandra Allan
• Impact of sub-lethal exposure to insecticides on vectors- Lee Cohnstaedt
• Traps, attractants, targets and insecticides- Chris Geden
For more information:

http://www.ars.usda.gov/saa/cmave/mfru
What about the future plans for the USDA mosquito and fly research program?
Objective 1. Determine components of behavior leading to resource location by mosquitoes (resting sites, sugar sources, mates, blood-meal hosts, oviposition sites).

Objective 2. Determine cues that regulate specific mosquito and tick behaviors:
- Resting behavior and mate location
- Sugar-seeking (*, **, +++)
- Host-finding (*, **)
- Oviposition site location (*, ***, ++)
- Tick attraction (*)
- Pesticide impact on behavior (+++)

Objective 3. Discover and characterize environmental predictors of invasive mosquito species distribution and associated pathogens:
- Develop accurate methods for assessing population density
- Characterize environmental predictors of spatial and temporal distribution
- Use ecologic and climatic factors to assess population densities (**, +, ++)
- Evaluate risk of exotic species and pathogen introduction (e.g., RVF)(**+, +, ++)

Objective 4. Develop larval surveillance methods for mosquitoes (*, **)
- Improved methods for rapid assessment of adult mosquito population density
- Spatio-statistical model for prediction of mosquito presence and abundance and for trap deployment
- National mosquito surveillance database and strategic model for detecting exotic vectors and Rift Valley fever (RVF) virus

New larval surveillance methods based on light and chemical preferences

New knowledge on resource location behaviors (resting, mating, sugar-feeding, host and oviposition site location) of mosquitoes
- New chemicals and non-chemical cues
- New lures (sugar, host and oviposition site) and more effective traps (host)
- More sensitive vector surveillance methods
- GIS-based model for resting site location.

Traps and lures for adult and larval mosquitoes for potential commercialization

Improved surveillance tools and strategies for biting arthropods
BITING NEMATOCERA: BIOLOGY & CONTROL

Research focused on developing knowledge and tools for improved control of biting Nematocera

**Objective 1.** Discover new control chemistries
- New toxicants (*, **) - Develop RNAi-based molecular pesticides

**Objective 2.** Develop new and improved control products & technologies
- Develop improved repellent-treatments for clothing (***)
- Characterize factors such as climate, vegetation, dust, soil, and fauna affecting adulticide performance in various environments (+, ++)
- Develop technologies to improve efficacy of residual insecticides (+, ++)
- Develop improved approaches to aircraft disinsection (+++)

**Objective 3.** Develop new strategies based upon attractants, repellents, and inhibitors
- Conceive and test new methods of vector control using behavior-modifying chemicals
  - Develop a “push-pull” system using behavior-modifying chemicals in baited trapping systems (+, ++, †, ††)

**Objective 4.** Evaluate, optimize and standardize repellent bioassays
- Evaluate factors that influence outcomes of assays to optimize and standardize repellent bioassays (†††)

- Improve repellents for use on clothing
- Characterize environmental factors that affect adulticide efficacy in various environments
- Improve control products and technology for aerosol and residual insecticide applications
- Develop devices that prevent insects from entering aircraft
- Develop attractant-toxicant traps
- Develop “push-pull” baited trapping systems to control insect pests within a local area

Improved standardized repellent bioassays for EPA label registration of topically-applied repellent products

Improved control products, technologies, and assays for biting Nematocera

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* SCA with University of Florida
** Cooperative Research and Development Agreement (CRADA) with Chemtura Corporation
*** Reimbursable (R) with US Army-RDECOM (Natick)
† SCA with WRAIR
+++ SCA with Coachella Valley MCD
+++ R with US DOT

†† Non-funded Cooperative Agreement (NFCA) with Southwest Research Institute
††† Memorandum of Understanding (MOU) with US EPA

Dr. Ulrich Bernier
Lead Scientist

New larvacides, adulticides, and RNAi-based biopesticides
**Objective 1:** Develop better tools for surveillance and risk assessment
- Feeding behavior/nutritional ecology of house flies.
- Identify/develop stable fly attractants for traps. (*)
- Monitor introduction of exotic *Stomoxys* spp. into the southeastern US.

**Objective 2:** Develop more efficient IPM methods
- Salivary gland hypertrophy virus (SGHV) for a microbial bait for control of house flies; interaction of SGHV and chemical treatment method for fly control.
- Biology and impact of diapriid parasitoids on immature stable flies and house flies. (*)

**Objective 3:** Test behavior-altering methods for control of stable and house flies
- Evaluate ARS stock of repellent compounds for stable flies on livestock. (**)  
  - Response of house flies to selected surfaces with and without addition of behavior-altering chemicals.
- Insecticide-treated visual targets to kill dispersing house flies.

**Objective 4:** Role of house flies in the dissemination of bacterial pathogens
- Determine the role of house fly-contaminated feed in the transmission of *Salmonella enteriditis* to chickens. (***)
- Identification of key nutritional attractants of house flies.
- New chemical lures for stable fly traps.
- Trapping data to determine risk of introduction of exotic *Stomoxys* spp. in the southeastern U.S.
- Virus-based baits to control house flies.
- Diapriid parasitoids ready for production by commercial insectaries to manage immature stable flies and house flies.
- New stable fly repellents for use on livestock.
- Behavior-altering chemicals/surface combinations to repel, attract, and/or kill house flies.
- A new insecticide treatment method for perimeter protection from dispersing flies.

New and improved biologically-based IPM tools (baits, traps, parasites, treated surfaces) for monitoring and managing house flies and stable flies.

New knowledge on routes of pathogen transmission by flies in poultry houses.

*Agroecosystem Management Research Unit, Lincoln, NE.*
**Invasive Insect Biocontrol & Behavior Laboratory, Beltsville, MD.*
***Southeastern Poultry Research Laboratory, Athens, GA.*