USDA ARS Knipling-Bushland Livestock Insects Research Laboratory: Research Update

Presented by Robert J. Miller, Ph.D.
Research Entomologist
USDA ARS Cattle Fever Tick Laboratory, Edinburg TX

KBUSLIRL Appropriated Research Projects 2015-2019

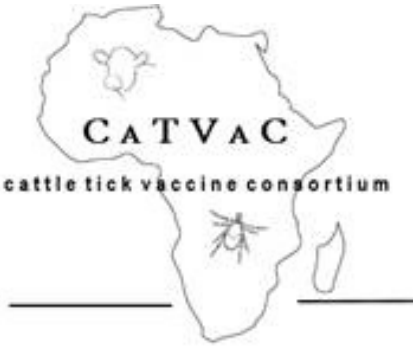
1. Genomics of Livestock Pests
   - Lead Scientist: Dr. Felix Guerrero
2. Cattle Fever Tick Eradication
   - Lead Scientist: Dr. Perez de Leon
3. Flies Associated with Livestock Production Systems
   - Lead Scientist: Dr. Kevin Temeyer
4. Innovative Technologies to Control Invasive Species that Impact Livestock
   - Lead Scientist: Dr. John Goolsby
5. Area-Wide Screwworm Eradication
   - Lead Scientist: Dr. Steve Skoda

Product Evaluation

- Acaricide efficacy testing
  - New products under development in partnership with a multi-national pharmaceutical company
  - Systemic, long-acting, 100% control of ticks
  - FY16, 2 field trials and a barn trial
  - Post-doc and Masters student
- Anti-tick vaccine efficacy testing
  - Multi-national pharmaceutical company
  - Testing new and established antigens for tick control
  - Implementation in the USDA APHIS VS eradication program and Puerto Rico

Cattle Tick Vaccine Consortium

- Bill and Melinda Gates Foundation
- To establish a closer collaboration between research groups that work on tick vaccines
- Establish a formal collaboration among the groups to facilitate exchange of antigens and evaluation of candidate vaccine formulations in standardized experimental models



Puerto Rico Project Update

Adalberto Perez de Leon
Fred Soltero
José Urdaz
Robert J. Miller

Puerto Rico (Potential Technologies for CFTEP)

Objective: Develop a safer and sustainable tick control management program for the island.

Methods: Epidemiological survey followed by treatment. Main effort against ticks. Fly and internal parasite control needed for holistic strategy.

Products Used:

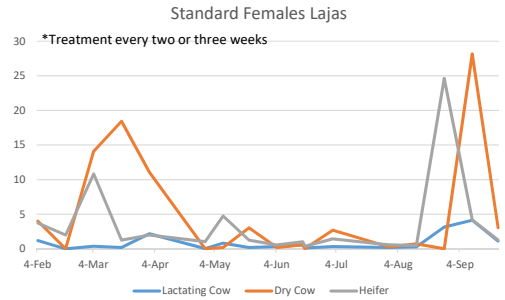
Ear Tags
Tolfenpyrad 15% Labeled for fly control, but is active against ticks.
XP-820
Abamectin 8%. Labeled for fly control and aids in the control of *R. microplus*

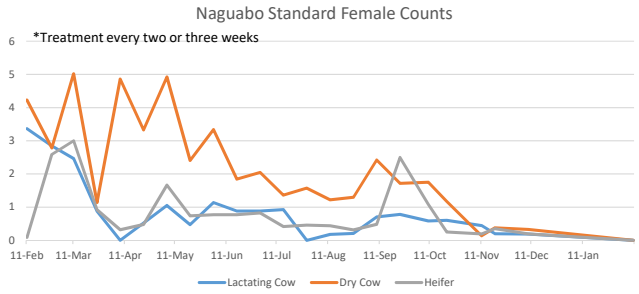
Essential Oil
Plant Essential Oils. Killed 100% of larvae and engorged adult ticks in laboratory bioassays when applied at the label rate.

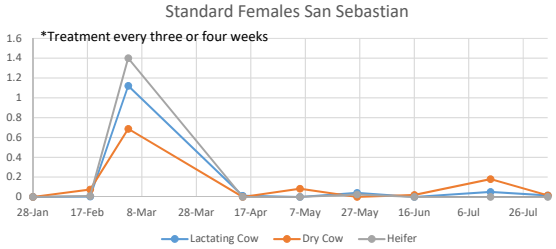
Diflubenzuron
Diflubenzuron. Chitin inhibitor. Labeled for fly control. Active against ticks.

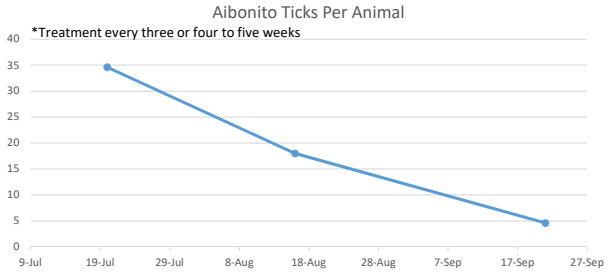
Eprinomectin Pour-on
Eprinomectin. Killed 95% of ticks in laboratory stall and field trials. (Davey et al. 2002 and Aguirre et al. 2005)

Results









Treatment Model for Puerto Rico

- Vaccinate all cattle against ticks
- Rotate macrocyclic lactone and essential oil applications every three weeks until ticks are eradicated
- Stop treatments monitor cattle for re-infestation
- Re-treat as needed
- Rotate ear tags or feed through diflubenzuron for fly control

Studies for FY17

- Vaccine synergies
  - Vaccine + acaricide
  - Vaccine + Babesia infection

National Tick Control Program for Puerto Rico

- Mandatory program vaccination + acaricide
- +600,000 vaccine doses requested over the next three years
- USDA APHIS VS to support through vaccination and animal treatments

COMMONWEALTH OF PUERTO RICO
Department of Agriculture

October 14, 2016

Suman Mahan
Associate Director
GD&O, VMRD, Zoetis
suman.mahan@zoetis.com

According to PRDA surveys and other data provided by cattle farmers, state, federal, and private veterinarians, tick infestation PR is a very serious concern. These ectoparasites (R.microplus) are responsible for what is known as "cattle tick fever" or babesiosis. These diseases are causing millions of dollars in losses to our cattle industry (beef & dairy).

Throughout the years, ticks have developed resistance to pesticides and other control and eradication efforts set forth by the government and the private sector. With this in mind, Puerto Rico Department of Agriculture (PRDA), Veterinary Services (VS) and Agricultural Research Services (ARS) have joined to develop and integrated program for the control and eradications of ticks. PRDA and ARS have reached an agreement, whereby, PRDA spent one million dollars ($1,000,000) for the research of alternative methods of controls and eradications of ticks. The result of this research is a promising new tool: Tick vaccine (immunomodulatory) BM-86 developed by Zoetis.

Implementation of this program is mandatory and island-wide, as ordered by the Secretary of Agriculture, Dra. Myrna Comas Paglin.

Below, please find and estimate of the number of vaccine units needed for the first three (3) years.

First Year

To vaccinate at least 45,000 head of cattle (beef & dairy). This means the acquisitions of 135,000 vaccine units plus the implementation of other means of tick control (essential oils, pastures, perimeter fence repair).

Fernández Juncos Avns., Stop. 19 ½ Santurce.
PO BOX 10163, San Juan, PR 00908-1163
Tel (787) 721-2120 Fax (787) 723-8512

DEPARTMENT OF AGRICULTURE

Research Update

John Goolsby, Ph.D.
Research Entomologist

Contributions:
Don Thomas, Weste Osbrink, Beto Perez de Leon,
Dave Hewitt, Greta Schuster, Poncho Ortega

US Dept of Agriculture, Agricultural Research Service, Cattle Fever Tick Research Laboratory, Edinburg, TX;
USDA-ARS Knipling Bushland U.S Livestock Insects Research Laboratory, Kerrville, TX;
Texas A&M Kingsville

Pathogenic Landscape

Infested hosts

River willows

Cattle fever tick

1. Arundo and Guineagrass enhance survival of tick
2. Transition back to native vegetation--better biological barrier to ticks

Racelis, A.E., R. B. Davey, J. A. Goolsby, A. A. Pérez de León, K. Varner, and R. Duhaime. 2012. Facilitative ecological interactions between invasive species: *Arundo donax* (Poaceae) stands as favorable habitat for cattle ticks (Acari: Ixodidae) along the US-Mexico border. Journal of Medical Entomology 49: 410-417.

Exotic Weeds in Cattle Fever Tick Quarantine Zone

Arundo donax
Giant reed

Tamarisk spp.
Saltcedar

Megathrysus infestus
Guineagrass

Candidate Biological Control Agents

Arundo wasp

Tetramesa romana
Arundo wasp
Hymenoptera: Eurytomidae
larvae feed on stems & side shoots (adult stingless)
Released April 29, 2009

Arundo scale

Rhizaspidiotus donacis
Arundo scale
Homoptera: Diaspididae
scale insects feed on roots and side shoots
Released Dec. 17, 2010

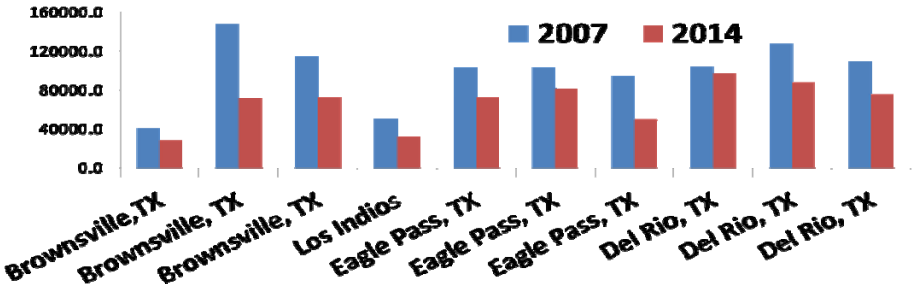
Arundo leafminer

Lasioptera donacis
Arundo leafminer
Diptera: Cecidomyidae
Larvae are leaf sheath miners causing defoliation
Release Sept 2016

Arundo fly

Cryptonevra spp.
Arundo fly
Diptera: Chloropidae
fly larvae feed on new stem shoots
In testing

Impact of the biological control program on *Arundo donax* biomass



- **22 % reduction in *A. donax* biomass between Del Rio and Brownsville**
- **2.5 million tons of *A. donax* removed**
- **6000 acre ft of irrigation water conserved per year**
- **Visibility into stands from 2m to 12m**
- **Ecological transition back to native vegetation beginning**

John A. Goolsby, Patrick J. Moran. Alexis E. Racelis, Kenneth R. Summy, Maricela Martinez Jimenez, Ronald D. Lacewell, Adalberto Perez de Leon, & Alan A. Kirk 2015. **Impact of the biological control agent, *Tetramesa romana* (Hymenoptera: Eurytomidae) on *Arundo donax* (Poaceae: Arundinoideae) along the Rio Grande River in Texas. Biocontrol Science & Technology (In Press).**

Tick Bot

FEASIBILITY OF A TICK BOT

MANUVERABILITY IN SOUTH TEXAS SCRUB PASTURE HABITAT

MATERIALS FOR DRAGS

FUNCTIONALITIES OF THE TICK BOT

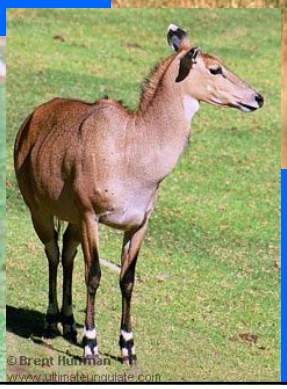
- DISRUPT LARVAL CLUSTERS
- DETECT TICK LARVAE
- ACARACIDE APPLICATION

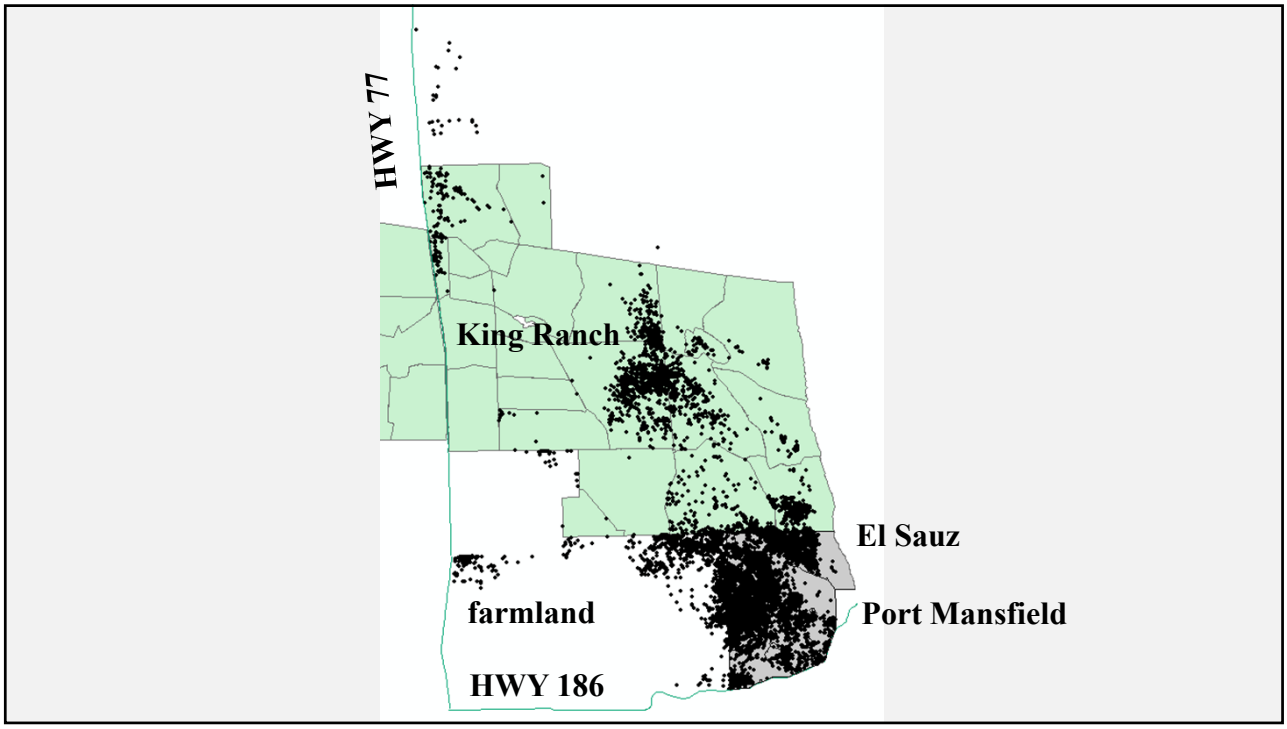
Dr. Don Thomas

UAV - Drones

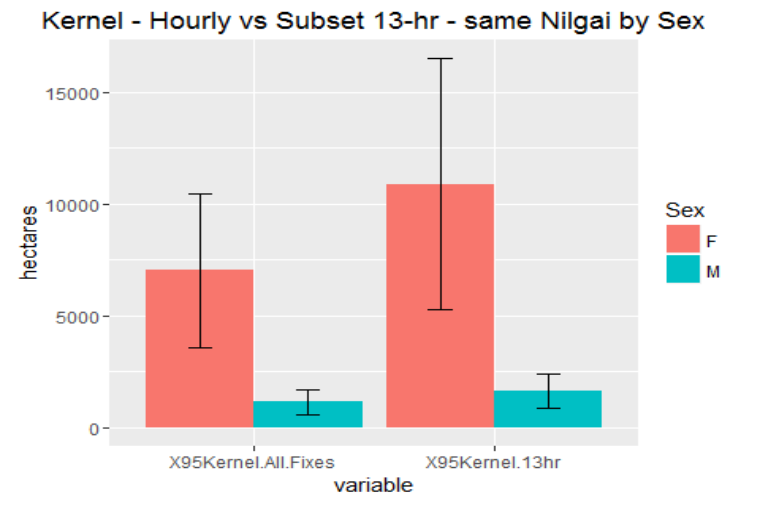
Nilgai Biology

- Members of the Bovidae
- Excellent hosts of cattle fever ticks
- Large home ranges





Females have > home range



Nilgai Lure Study

- Nilgai attractants would be useful for developing CFT treatments
- Nilgai form communal latrines
- Nilgai prefer to make latrines on offal
- Testing synthetic offal (Screw worm lure, and bovine volatile fatty acids (VFA)

Nilgai defecating at Screwworm Lure Site

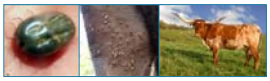
Tick Parasitoid

- *Ixodiphagous mysorensis*
- Tick parasitoids native to Asia in native range of
- *R. microplus* and nilgai

Biological control of cattle fever ticks

J. Goolsby[1], F. Guerrero[2], J. Kashefi[3], L. Smith[3], A. Racelis[4], D. Amalin[5], M.J. Flores[5], N. Abes[5], K. Verly[5], N. Singh[6], P. Azhahianambi[7], A. Jesudasan[8], P. DeBarro[9], A. Sheppard[9], K. Wyckuys[10], J. Liu[11], A. Schwartz[12], H. Hasel[13], K. Varner[13], & A. Pérez de León[2]
[1]United States Dept. of Agriculture, Agricultural Research Service, Knipling Bushland U.S. Livestock Insect Pest Research Laboratory, Cattle Fever Tick Unit, Edinburg, TX, john.goolsby@ars.usda.gov; [2]USDA-ARS Knipling Bushland U.S. Livestock Insect Pest Research Laboratory, Veterinary Pest Genomics Center, Kerrville, TX, [3]USDA-ARS European Biological Control Laboratory, Montpelier, France; [4]Univ. of Texas, Rio Grande Valley; [5]De La Salle University, Manila, Philippines; [6]Dept. of Veterinary Parasitology, Guru Angad, Dev Veterinary and Animal Sciences Univ., Punjab, India; [7]Dept. of Veterinary Parasitology, Madras Veterinary College, TANUVAS, Chennai, India; [8]Madras Christian College, Chennai, India; [9]CSIRO Health & Biosecurity, Brisbane & Canberra, Australia; [10]International Center for Tropical Agriculture, Hanoi, Vietnam; [11]Hebei Normal University, Shijiazhuang, Hebei, P.R. China, [12]Texas Animal Health Commission, Austin, TX, [13]USDA-APHIS, Cattle Fever Tick Eradication Program, Laredo, TX.

Cattle Fever Ticks



The southern cattle fever tick, *Rhipicephalus microplus* has several important hosts including domestic cattle, whitetail deer and Indian nilgai antelope. Biological control could potentially reduce populations of tick in Mexico and reduce the risk of invasion into the U.S. along the Rio Grande. Biological control could be integrated with current cattle fever tick eradication programs.

Wildlife Hosts of

White-tailed deer and other exotic wildlife can be hosts of CFT and treatment of tick infestation is difficult. Nilgai (native to India) are highly mobile animals and have the potential to spread ticks beyond the border with Mexico and within temporary quarantine zones in Texas. Currently, no treatment is available for CFT on nilgai.

Exploration in Native Range

Fig. 1. Native and introduced ranges of cattle fever ticks (*Rhipicephalus* spp.) Red = introduced range of *R. microplus*, Pink = *R. microplus* eradicated, Blue = native range of *R. annulatus*, Purple = introduced range, Green = native range of *R. microplus*, Yellow = native range of *R. australensis*. Stars = search locations for biocontrol agents.

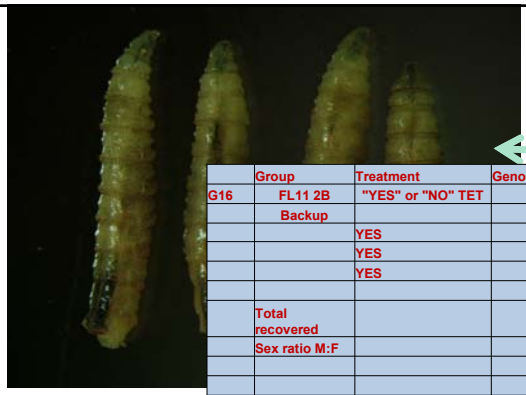
Potential Biocontrol Agents

These pictures show the tick parasitoid *Ixodiphagus hookeri*, a parasitoid of the tick, *Ixodes scapularis*. We hypothesize that a similar specialist parasitoid occurs in the native range of cattle fever tick. On right is *Ixodiphagus aethes*, a newly described species from India. Its tick host is unknown.

These pictures shows the predator mites *Anystis jabanica* and *Walstia australica* which are known to predate on *R. microplus* in Indonesia/Australia. On right is a steinernemid nematode which can be used as a tick biocides. Other CFT specialists may occur in the native range.

Tick Exposures to Collect Parasitoids Predation Questing LarvaeSoil-Dwelling Predators

USDA Agricultural Research Service

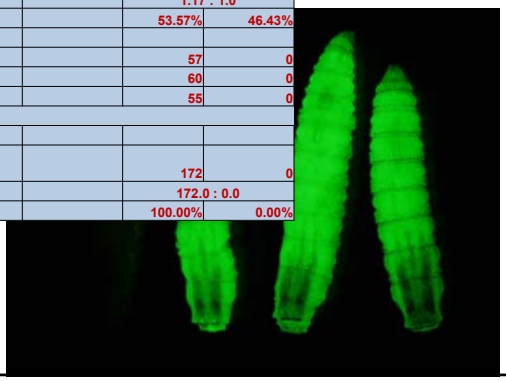


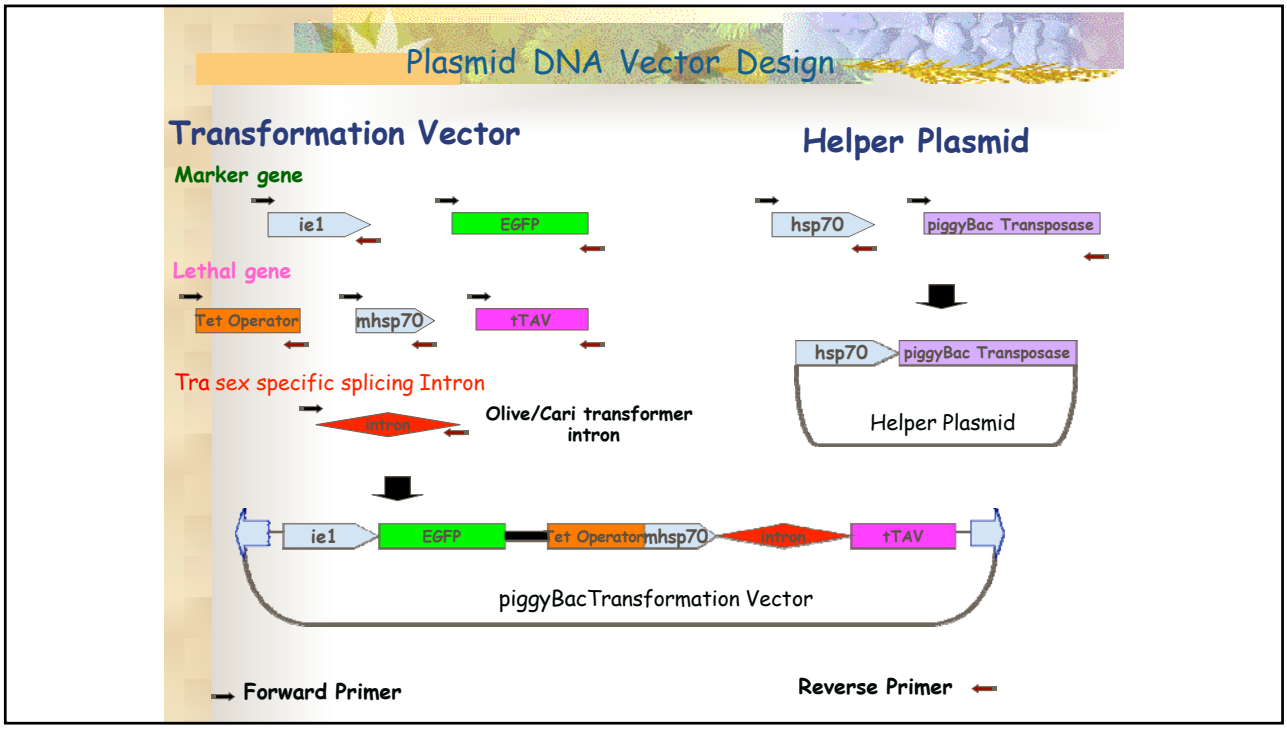
Transgenic screwworms

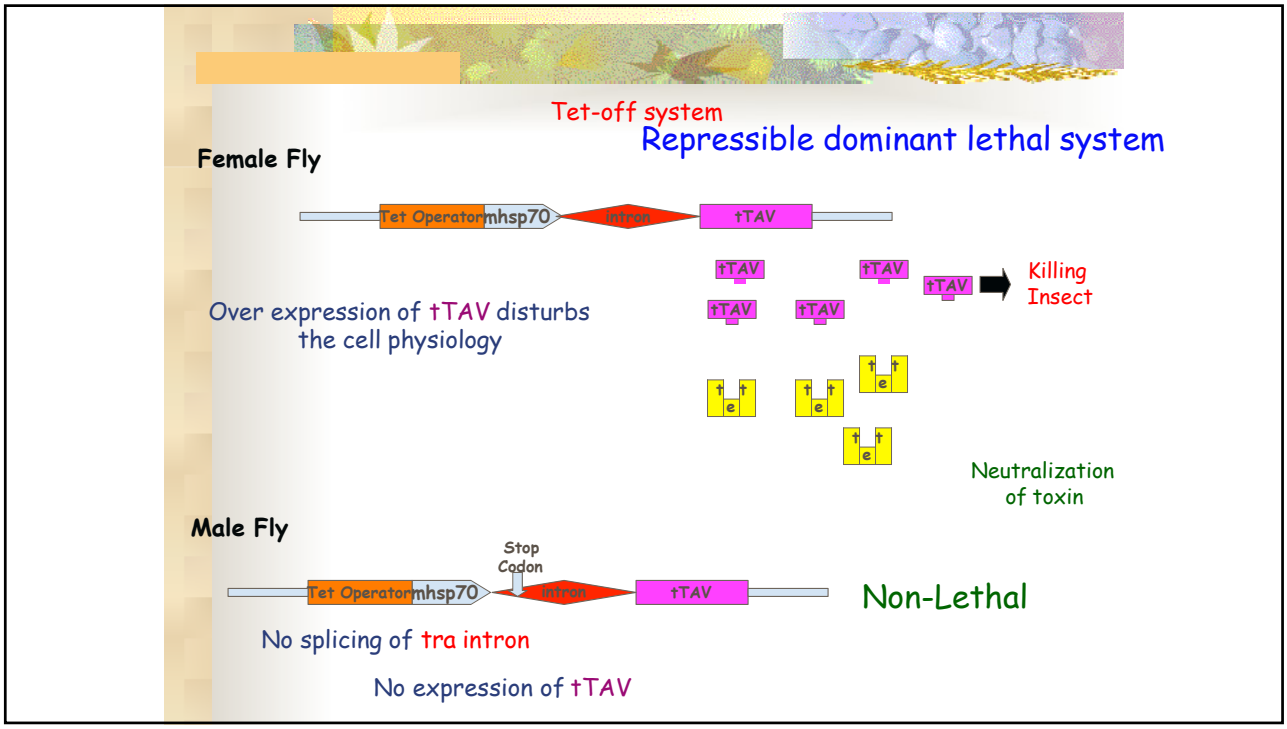
white light

	Group	Treatment	Genotype	N° of Male	N° of Female
G16	FL11 2B	"YES" or "NO" TET	WW		
	Backup				
		YES		40	38
		YES		46	38
		YES		34	27
	Total recovered			120	103
	Sex ratio M:F			1.17 : 1.0	
				53.57%	46.43%
		NO		57	0
		NO		60	0
		NO		55	0
	Total recovered			172	0
	Sex ratio M:F			172.0 : 0.0	
				100.00%	0.00%

Same screwv under light



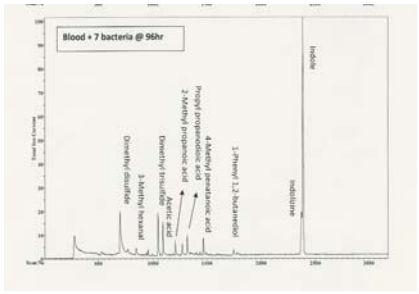

Plasmid DNA Vector Design
Transformation Vector
Helper Plasmid
Marker gene
ie1
EGFP
Lethal gene
Tet Operator
mhsp70
tTAV
Tra sex specific splicing Intron
intron
Olive/Cari transformer intron
hsp70
piggyBac Transposase
Helper Plasmid
piggyBacTransformation Vector
Forward Primer
Reverse Primer

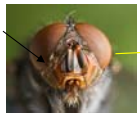

Tet-off system
Repressible dominant lethal system
Female Fly
Tet Operator
mhsp70
intron
tTAV
Killing Insect
Over expression of tTAV disturbs the cell physiology
Neutralization of toxin
Male Fly
Stop Codon
Non-Lethal
No splicing of tra intron
No expression of tTAV

Develop screwworm oviposition attractant.

Extraction and Identification

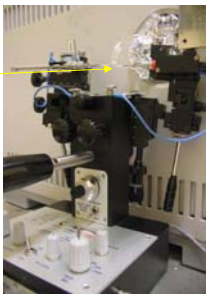
GC-MS Determination of Compounds from Volatiles Produced by 7 Species of Bacteria



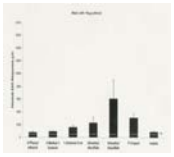


Fly Head

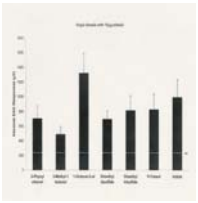
Electroantennography



EAG Responses to Selected Chemicals



Males



Females

Sequencing the genome of screwworms.

Assisting in the response to the screwworm outbreak in the Florida Keys. Key deer are severely impacted.

Thank you very much

Scratching for ticks in Puerto Rico