# Insecticide Resistance Testing at a Mosquito Control Program


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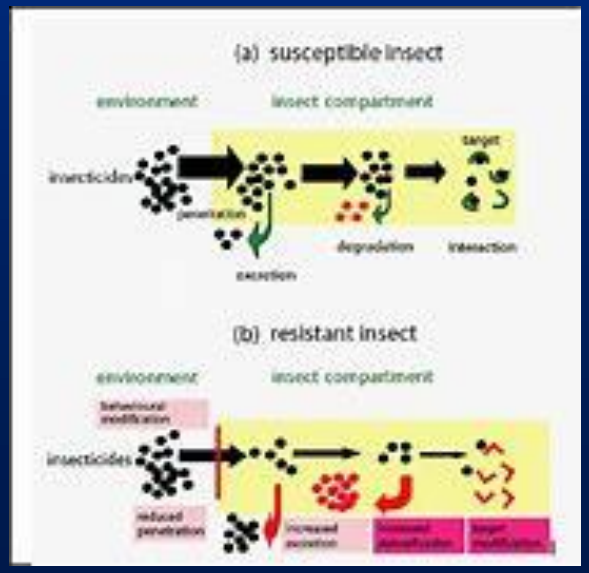
City of New Orleans Mosquito & Termite Control Board

# Types of resistance

1) Reduced penetration
2) Metabolic resistance (enzymes)
3) Change in target site
4) Behavioral resistance



## What is metabolic resistance?

**Metabolic resistance occurs when resistant mosquitoes develop enzymes that more rapidly detoxify pesticides, preventing the active ingredient from reaching its physiologic target.**

## What is target-site resistance?

**Target-site resistance is observed when the target of the pesticide on the mosquito is altered by a mutation**
**For example, mutations of sodium channel receptors produce resistance to pyrethroids, and mutations of the neurotransmitter acetylcholinesterase produce resistance to organophosphates and pyrethroids**

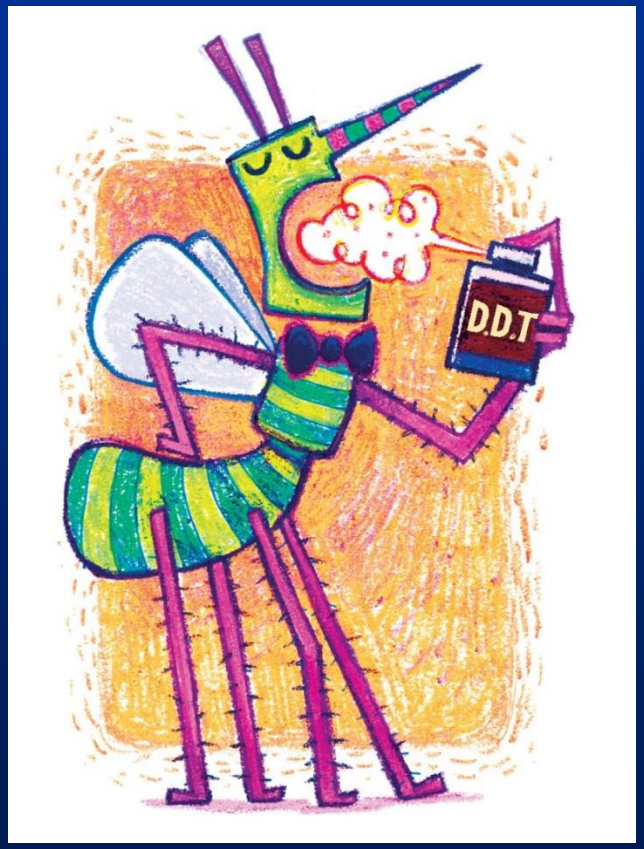
# Traditional Definition

"**Insecticide resistance**" describes the ability of strains of insects to survive "**normally**" lethal doses of insecticide, the ability having resulted from selection of tolerant individuals in populations exposed to the toxicant for several generations.

**The time to act is before resistance reaches this level.**

# Resistance Definition

- **Insecticide Resistance** is a genetic change in response to selection by toxicants that **<u>may impair control</u>** in the field.



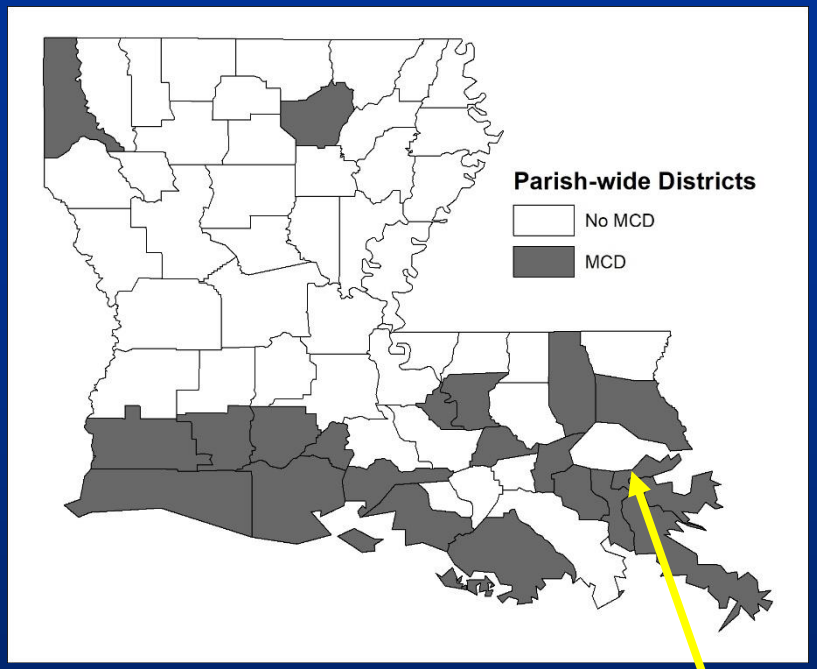
# How resistance is developed

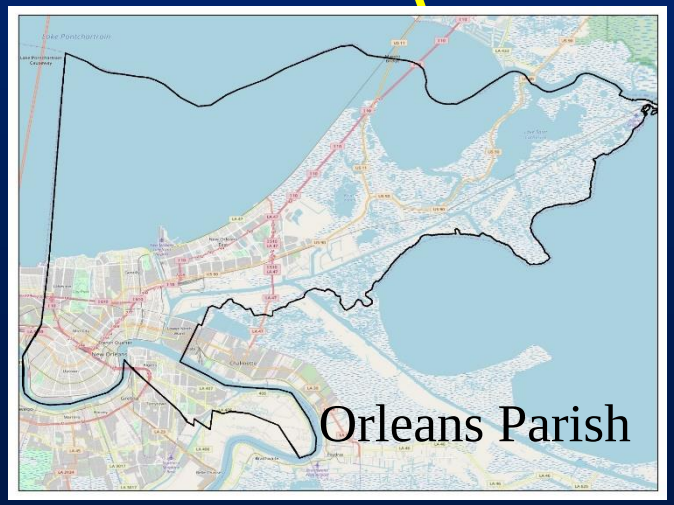
- single class of insecticide
- long-residual action
- slow-release formulation
- apply to all life states, all generations
- treat all habitat where pest occurs

# Mosquito Abatement Districts

- Out of 64 parishes in LA, we are one of 23 parish-wide mosquito abatement programs in Louisiana.

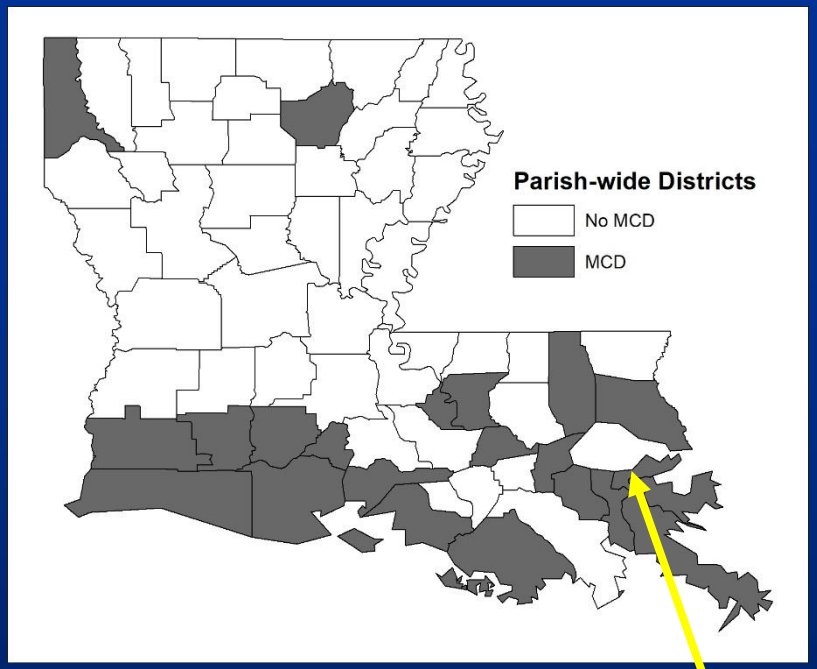
- Districts are managed independently and make resource allocation decisions to manage nuisance and mosquito disease vectors <u>within their parish jurisdictions.</u>

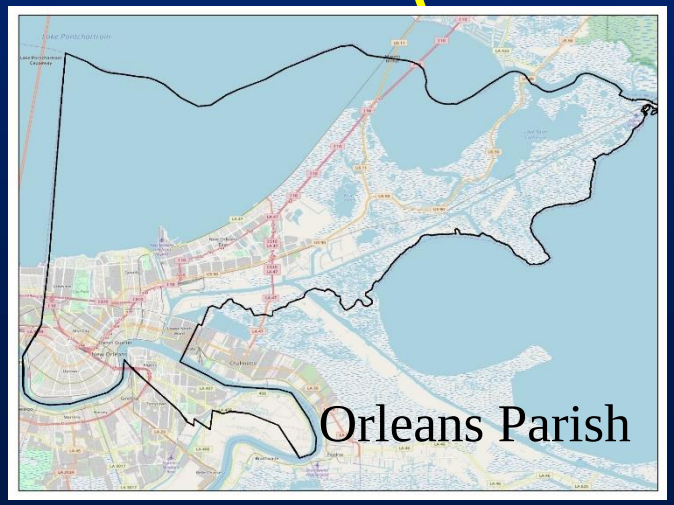


# Mosquito Abatement Districts

- Resource allocation and depth of program
  - spray truck, no surveillance
  - small programs with surveillance
  - medium sized public
  - Well funded (>7 million/yr) with significant capital assets
  - Multi-dimensional programs
    - Rodent control
    - General pest
    - Public works



**Mission**

Minimize the incidences of disease transmission, economic loss, and medical emergencies caused by pests (i.e. mosquitoes, rodents, termites).

**Action**

We achieve this mission by managing pest populations through a series of integrated approaches including surveillance, source reduction, biological control, sanitation, community education, and adult/larval abatement operations.

# Why We Control Mosquitoes?

- Nuisance
  - Quality of life
  - What species are considered nuisance?
- Mosquitoes of medical importance
  - Major disease risks:

West Nile Virus

- A virus that usually infects birds and transmitted by mosquitoes
- Occasionally infects humans and horses (dead end)
- Now endemic and cases occur each year

Dengue, chikungunya, and Zika

- A virus that infects human and transmitted by mosquito

*Culex quinquefasciatus*

*Aedes aegypti*

*Aedes albopictus*

# Control Strategies

- Chemical Program
  - Relies mainly on use of chemicals
  - May have limited surveillance

- Integrated Pest management Program
  - Uses Integrated Mosquito Management of which there are 8-9 components

# Integrated Pest Management (IPM)

EPA

Integrated Pest Management (IPM) is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices.

- IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment.

- This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment.

# Integrated Mosquito Management

Synergistic, ecosystem-based strategy that focuses on long-term suppression of pests or their damage through a combination of techniques, including biologic control, trapping, habitat manipulation, and chemical control.

Core includes:

1. Surveillance, mapping, and rational setting of action thresholds
2. Physical control through manipulation of mosquito habitat
3. Larval source reduction and adult mosquito control
4. <u>Monitoring for insecticide efficacy and resistance</u>

# IPM

Public Education and Community Outreach

Physical Control

Resistance Monitoring

Biological Control

Chemical Control

Disease Surveillance

Legal Actions

Mapping

Mosquito Sampling and Surveillance

# Integrated Pest Management

- Surveillance
- Source Reduction
- Biological Control Agents
- Larvicides
- Adulticides – “when not to spray”
- Public Education
  - Reduce conducive conditions
  - Eliminate containers holding water
  - Repellents, prevention

# Survey vs. Surveillance

- Survey – one-time gathering of inspection data to assess a situation

- Surveillance – a continuing process to monitor changes in mosquito populations
  - Indicates when control measures are needed
  - Monitors effectiveness of these measures
  - Resistance

# Traps for Adult Mosquitoes

- Identify the mosquitoes

# Why Mosquito Surveillance and Monitoring?

- Estimate Presence/Absence of Mosquito Vectors
- Estimate Population Size
- Public Health Concerns/Needs
  - Disease
- Control Application and Evaluation

# Why need to save the mosquito records

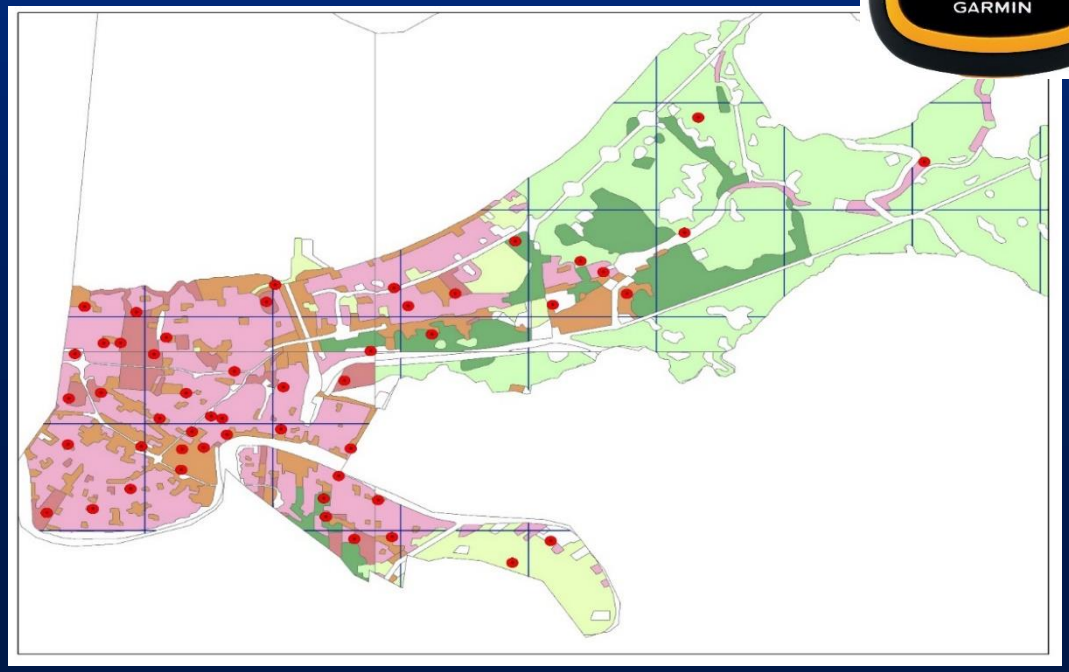
- Monitor spatial and temporal mosquito population dynamics and their relationship with virus transmission and disease incidence
- Establish what are the habitats of mosquitoes and their spatial and temporal dynamics
- Study the relationships between weather, mosquito populations, and disease
- Establish if vector control measures are effective, etc.

# Record Keeping

- Date of inspection, location & inspector
  - Pen & paper
  - Collection device (GPS, phone, laptop)
- Location description – address, intersection, grid, geographic features, GPS coordinates
  - Routine locations – numbered, named
  - Land use and land cover change
  - Demographic characteristics
- Importance–
  - Predict pattern of mosquito populations, must know the historical data
  - Target the temporal window of mosquito population peak
  - Designate resource allocation

# Mapping = Data Management (GIS/RS)

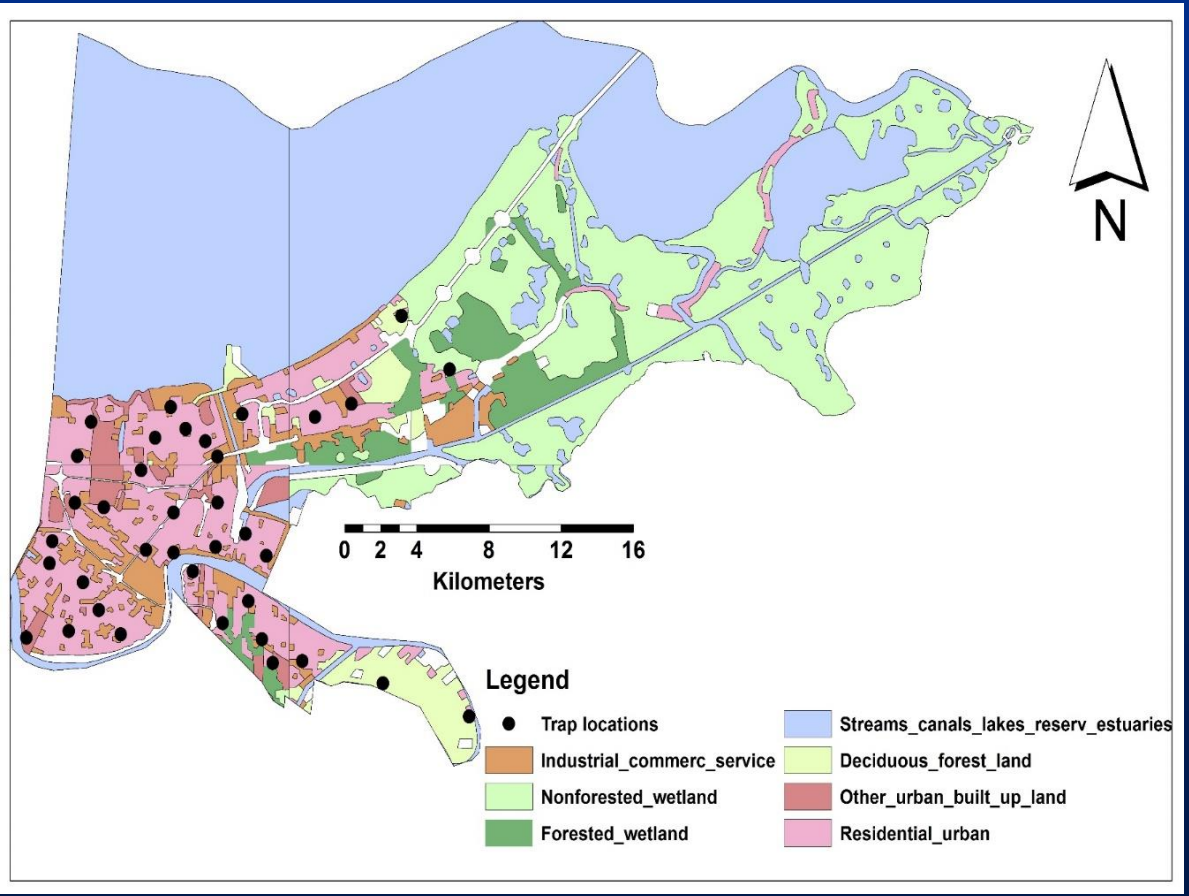
Can be as low tech as maps on the wall

Using GPS and GIS allows for data management and analysis as well as creates good records.

# GIS: Capturing Data

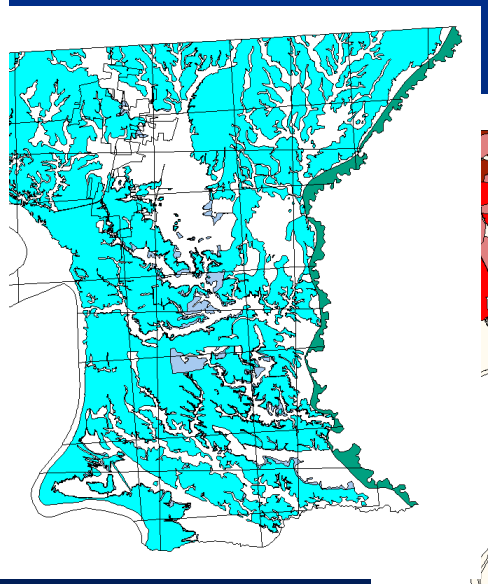
- Environmental Data
- Land use – land cover
- Surveillance data
- Control Data



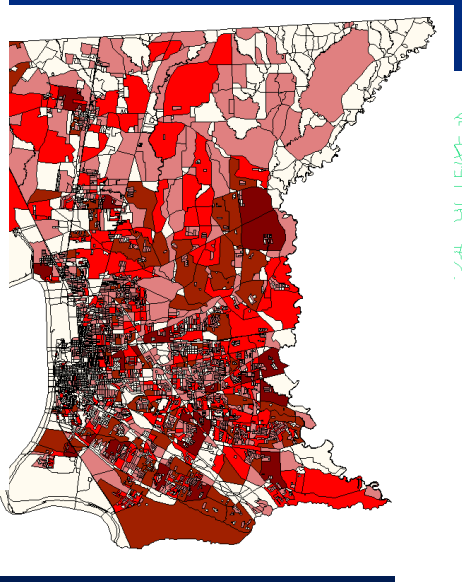
# GIS: Data Modeling

- Habitat suitability maps for adult/larval mosquito may be related to land use-land cover, meteorological, socioeconomic variables

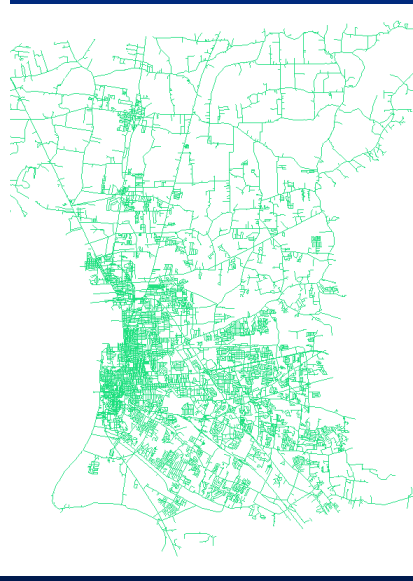
Adult mosquito records



Meteorology



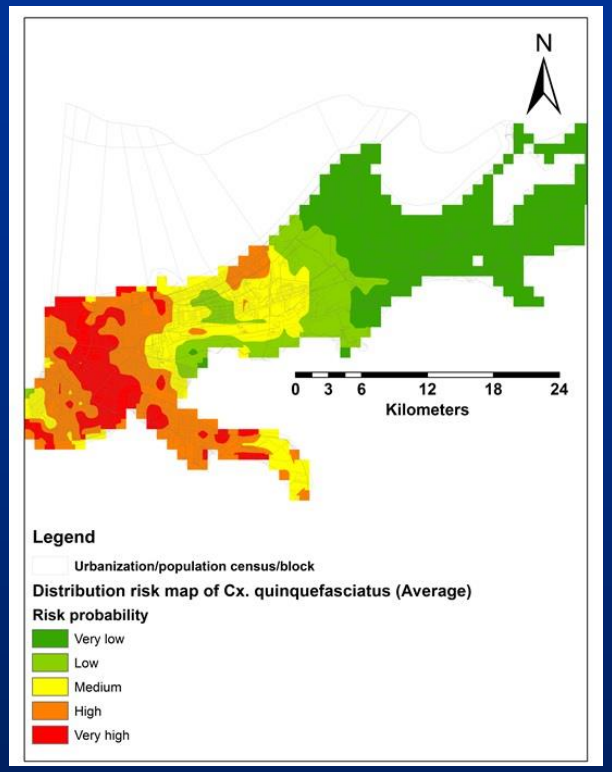
Socio-economic

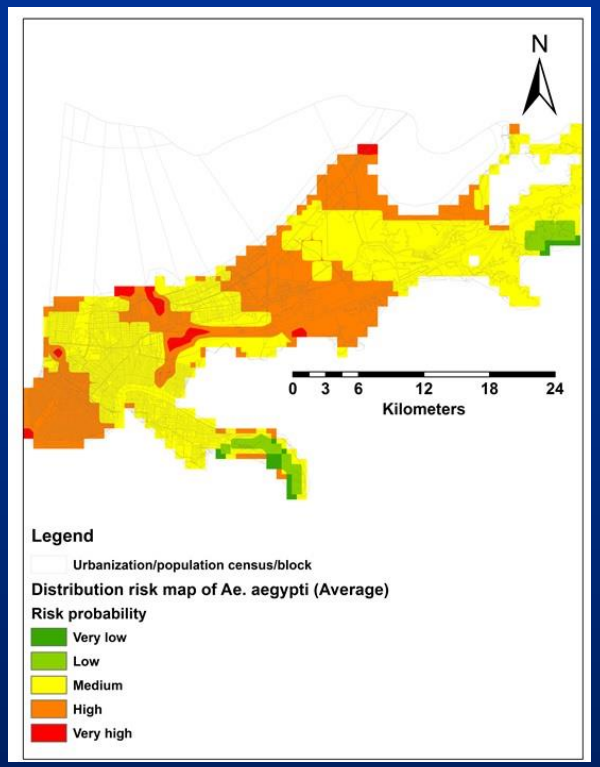


Vegetation

# GIS: Data Output

(Mapping and Prediction of Vector/Disease Distribution)




- 2015, Cx. q and *Aedes* surveillance data
- Model the area with increased risk of bites- increased transmission
- Most models rely on only on presence/absence of mosquitoes
- This model takes into account human pop density

Sallam, M.F., et al., 2017. IJERS

# INTEGRATED PEST MANAGEMENT

- Larvicides
- Adulticides


MOSQUITO
CONTROL


BU2145
West Nile Mosquito Control
240-6349

# ULV hand fogger and portable mist blowers

- Pyrethroids/pyrethrum



- Portable mist blowers
- Large droplet size

- ULV
- Droplet size 10-46 microns

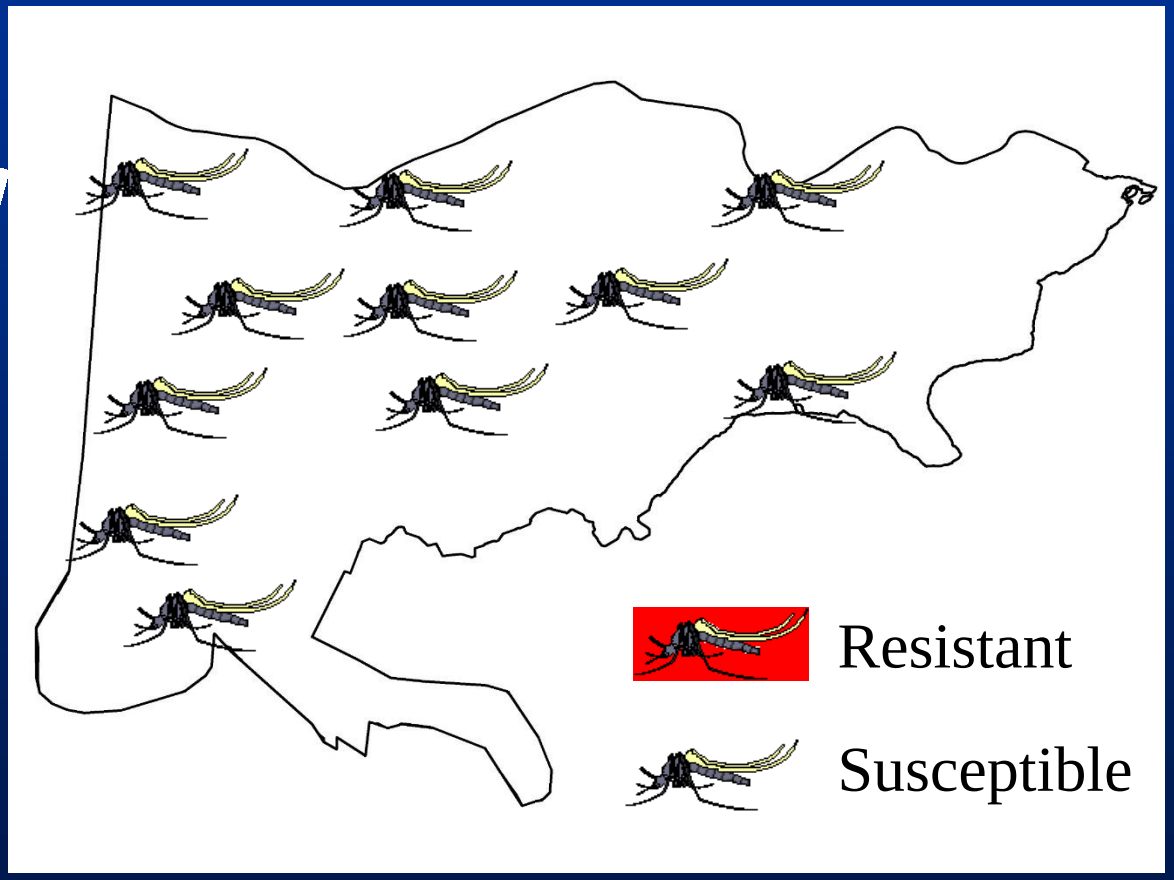
# Backyard Treatments

- Backyard treatments
  - Barrier/residual
    - Bifenthrin
    - λ-cyhalothrin
  - Non-residual
- Equipment
  - Backpack sprayers
  - Hand-held foggers

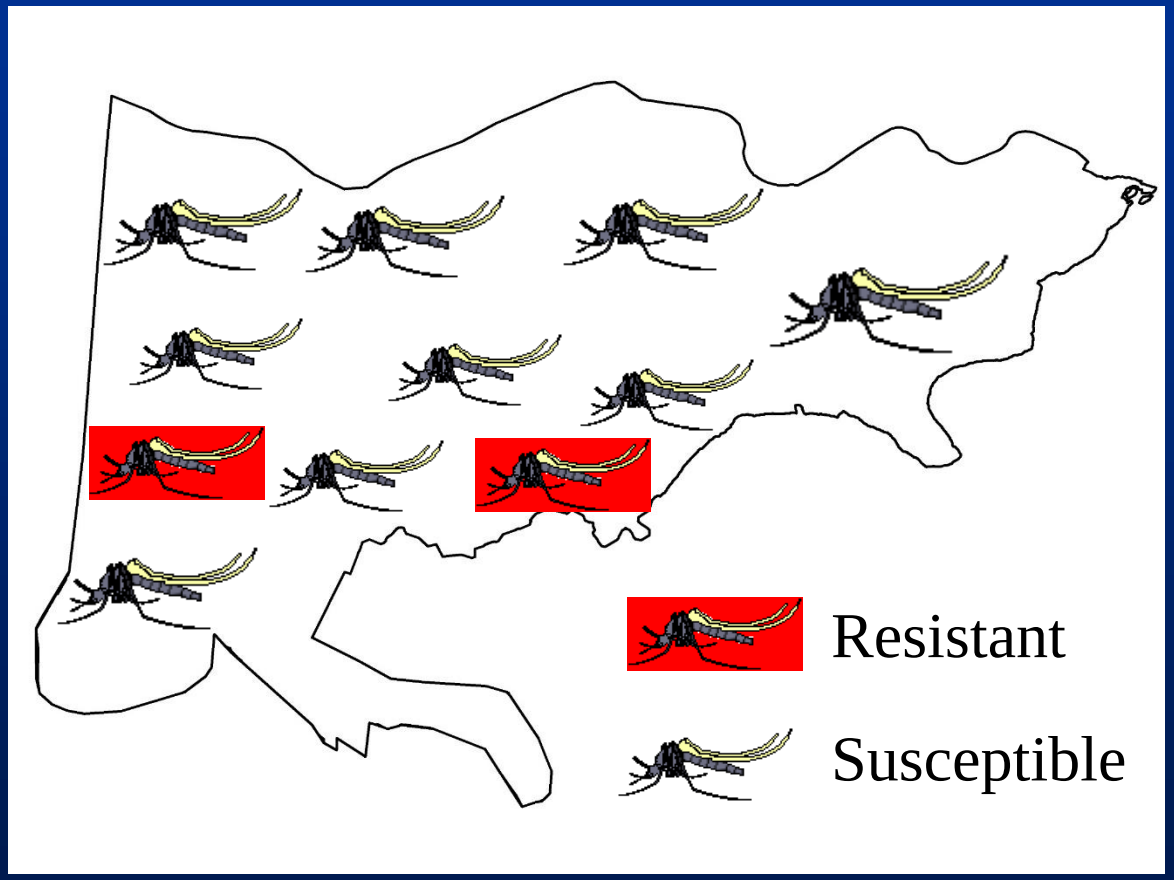
# Misting systems

Over the counter,
Consumer market

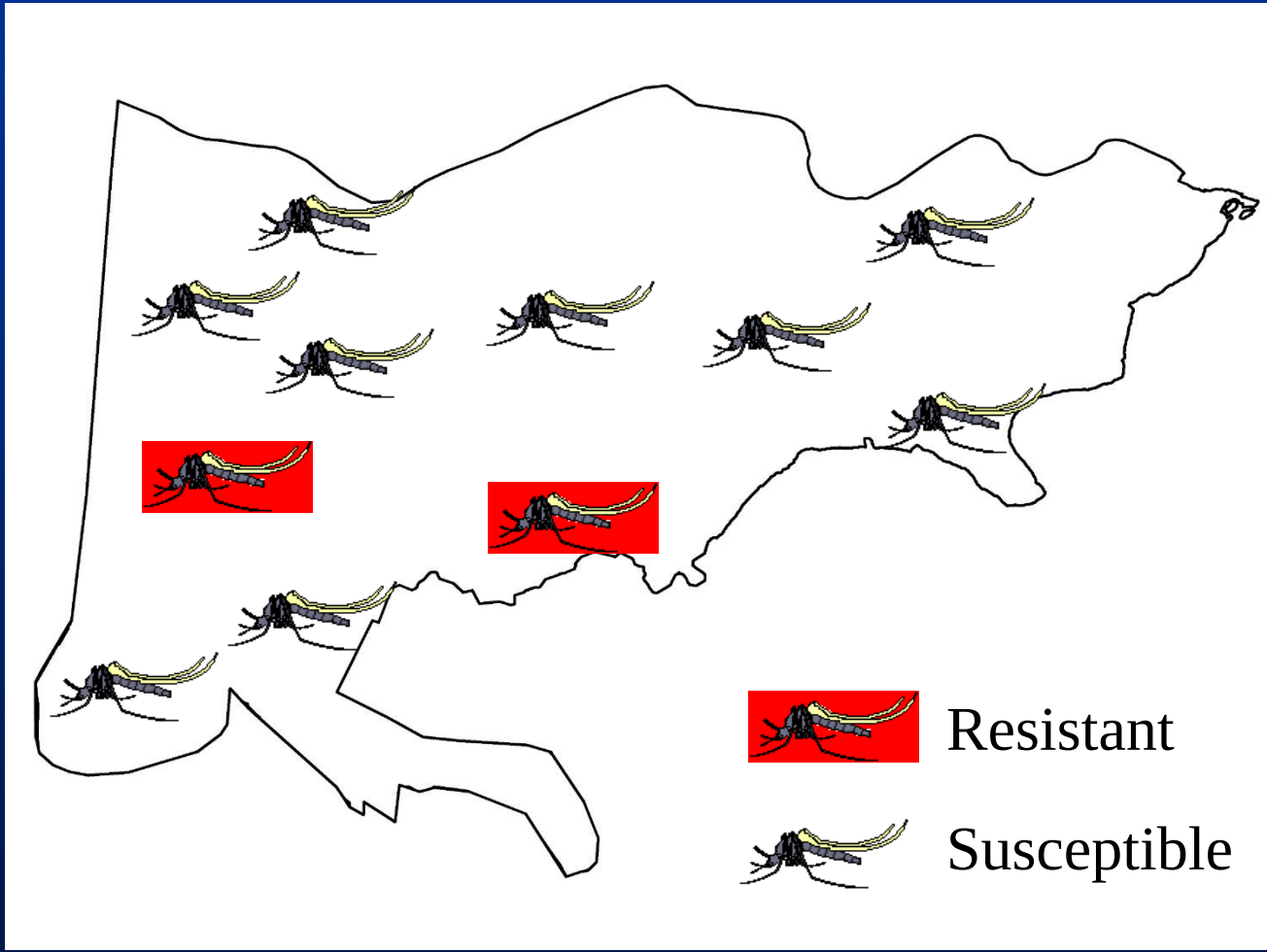
# Spray NOLA Mosquito Populations

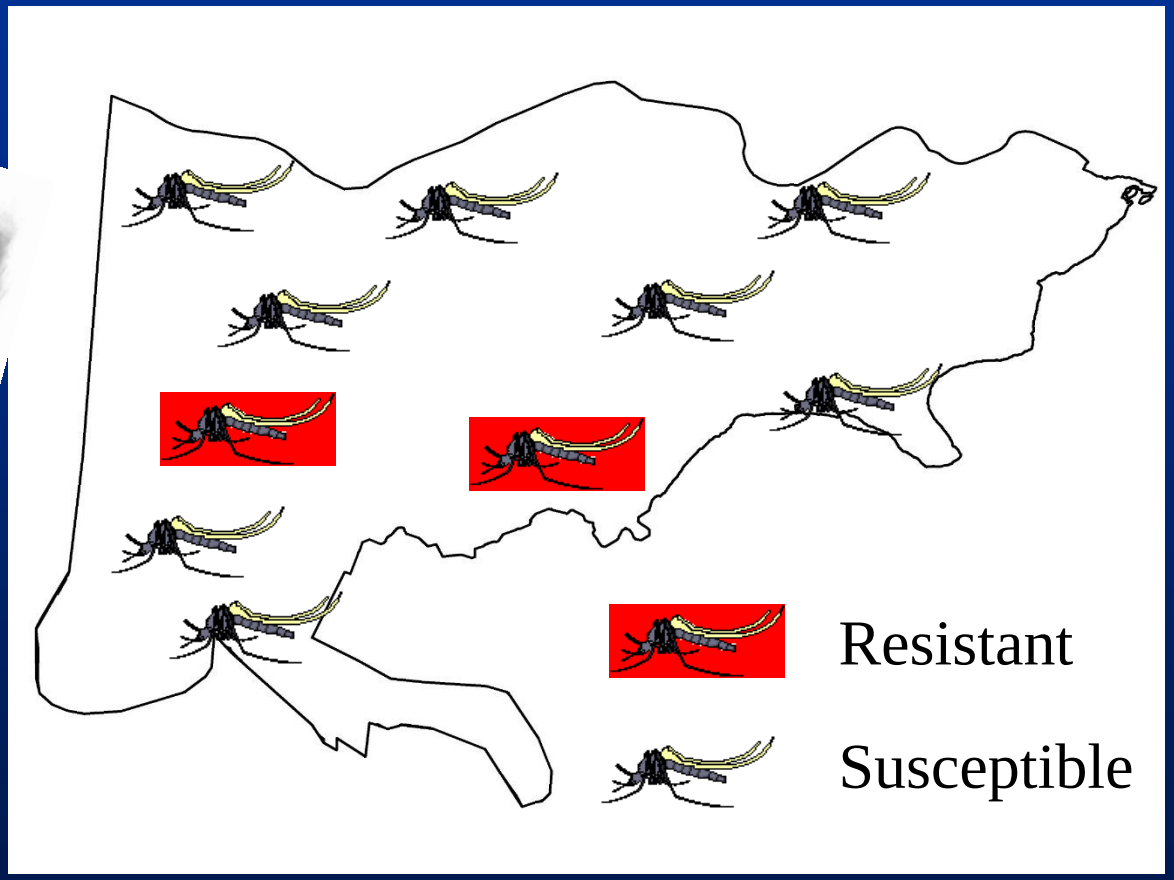
# Some survive with selected population



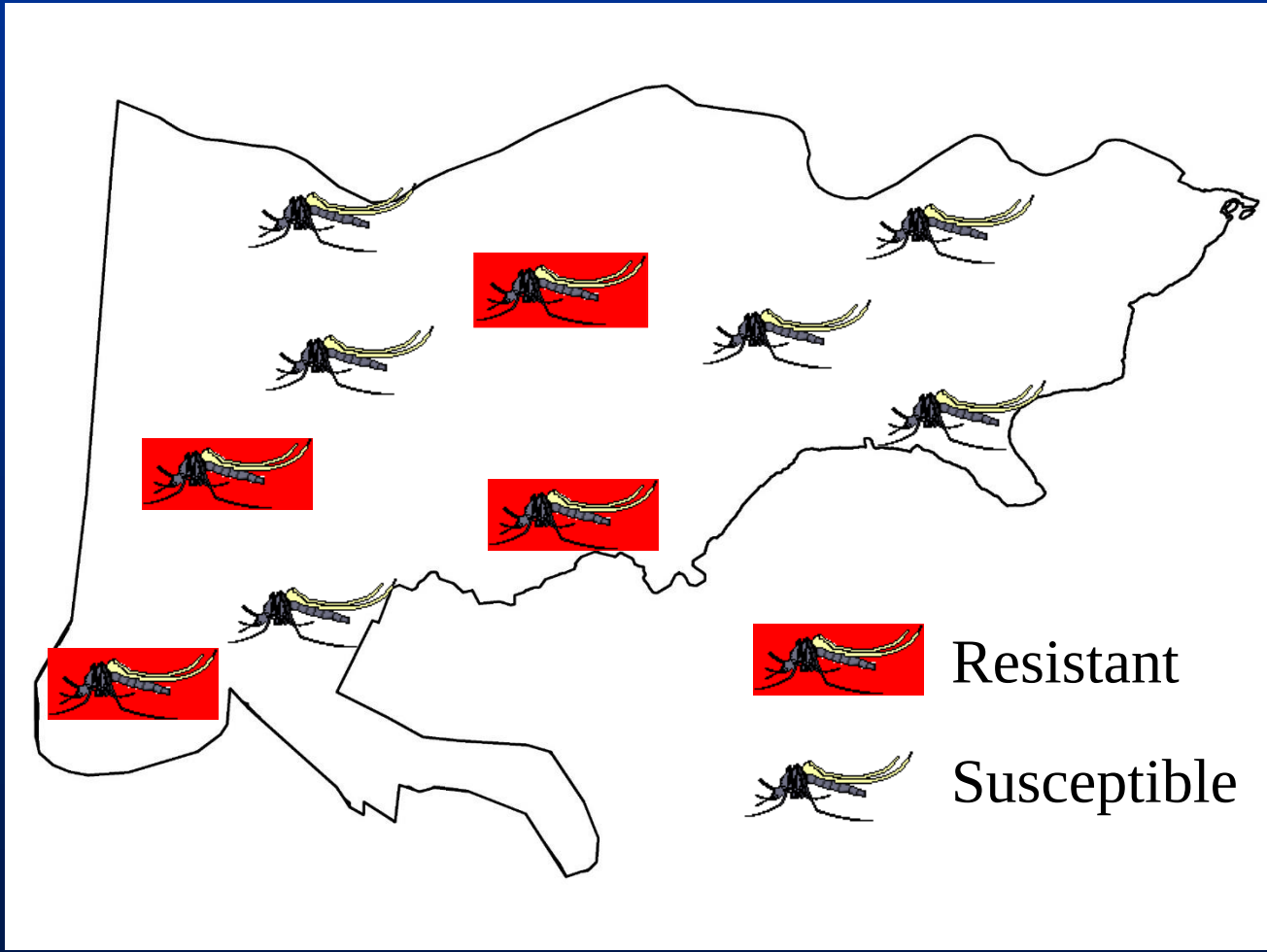
# Offspring of the survivor



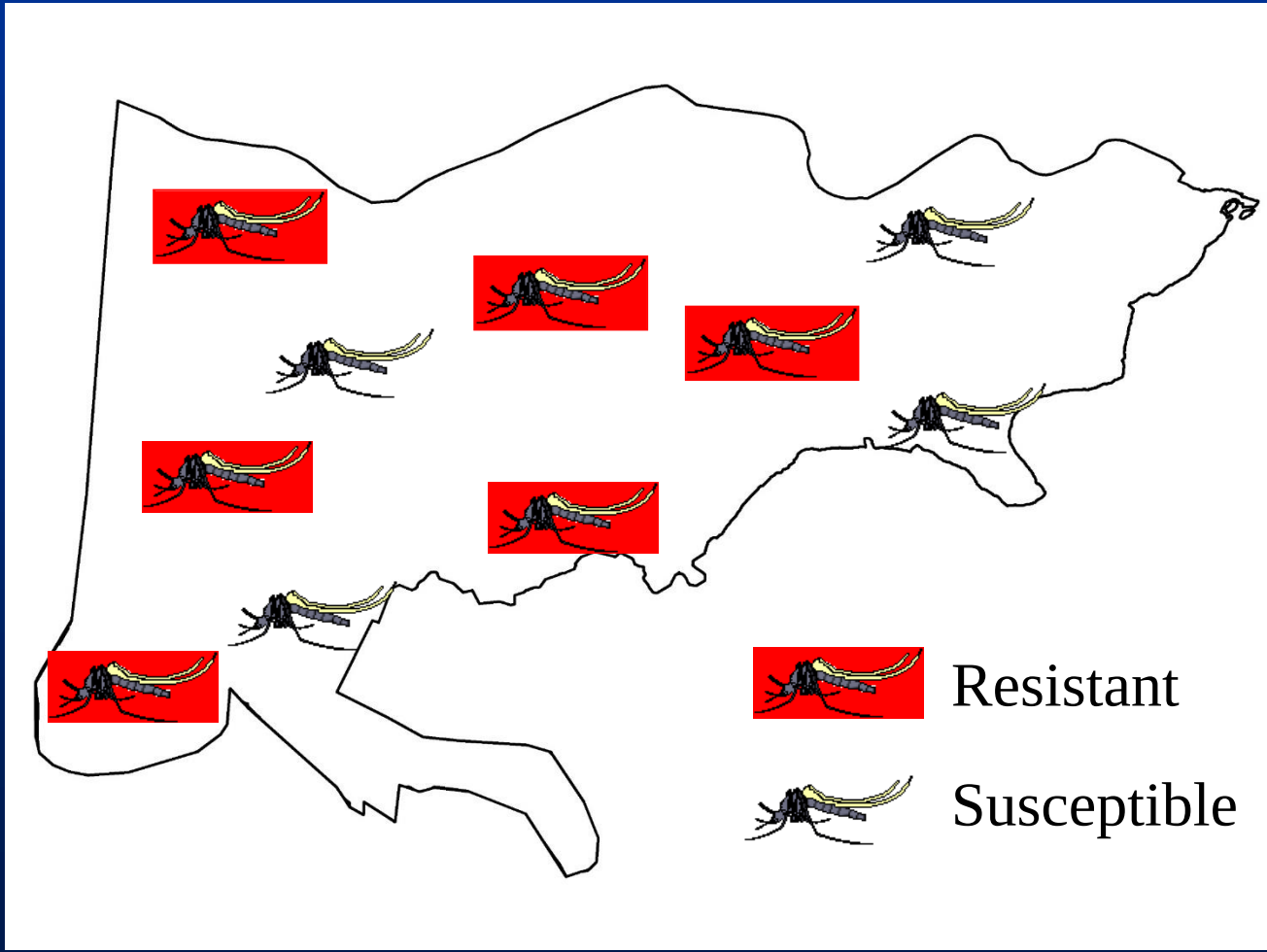
# Spray Again

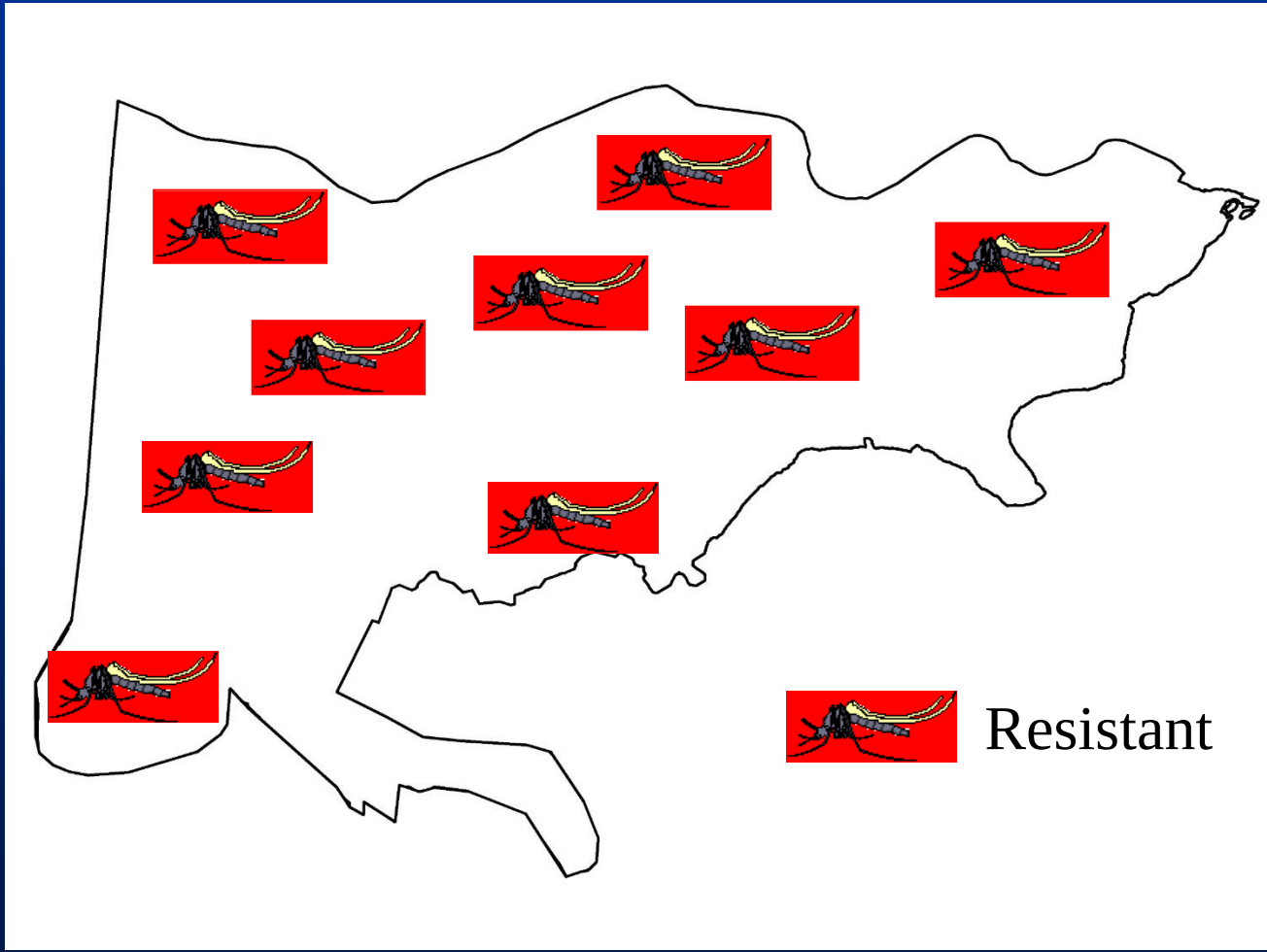
# More survivors



# More resistance in mosquito population



# …Resistant population!



# How resistance is developed

- single class of insecticide
  - Watch out for cross-resistance
- long-residual action
- slow-release formulation
- apply to all life states, all generations
- treat all habitat where pest occurs

# Types of Resistance

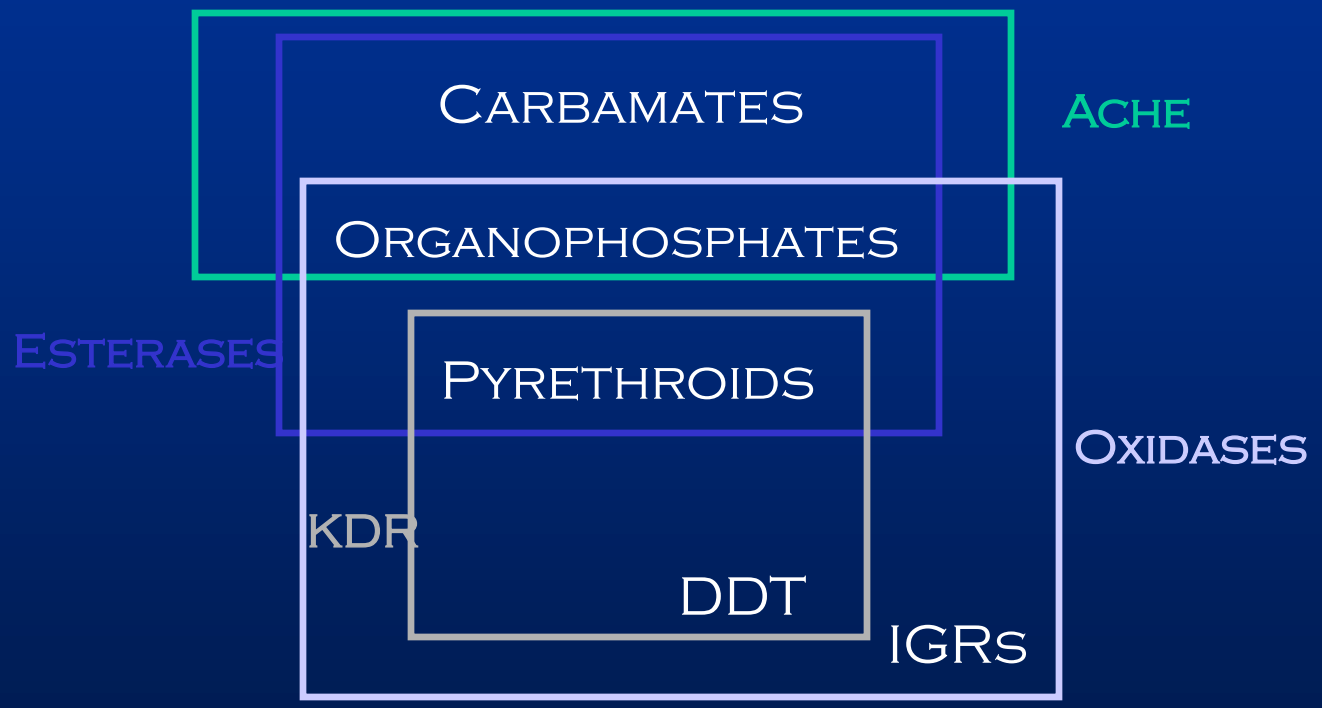
**Cross-resistance**

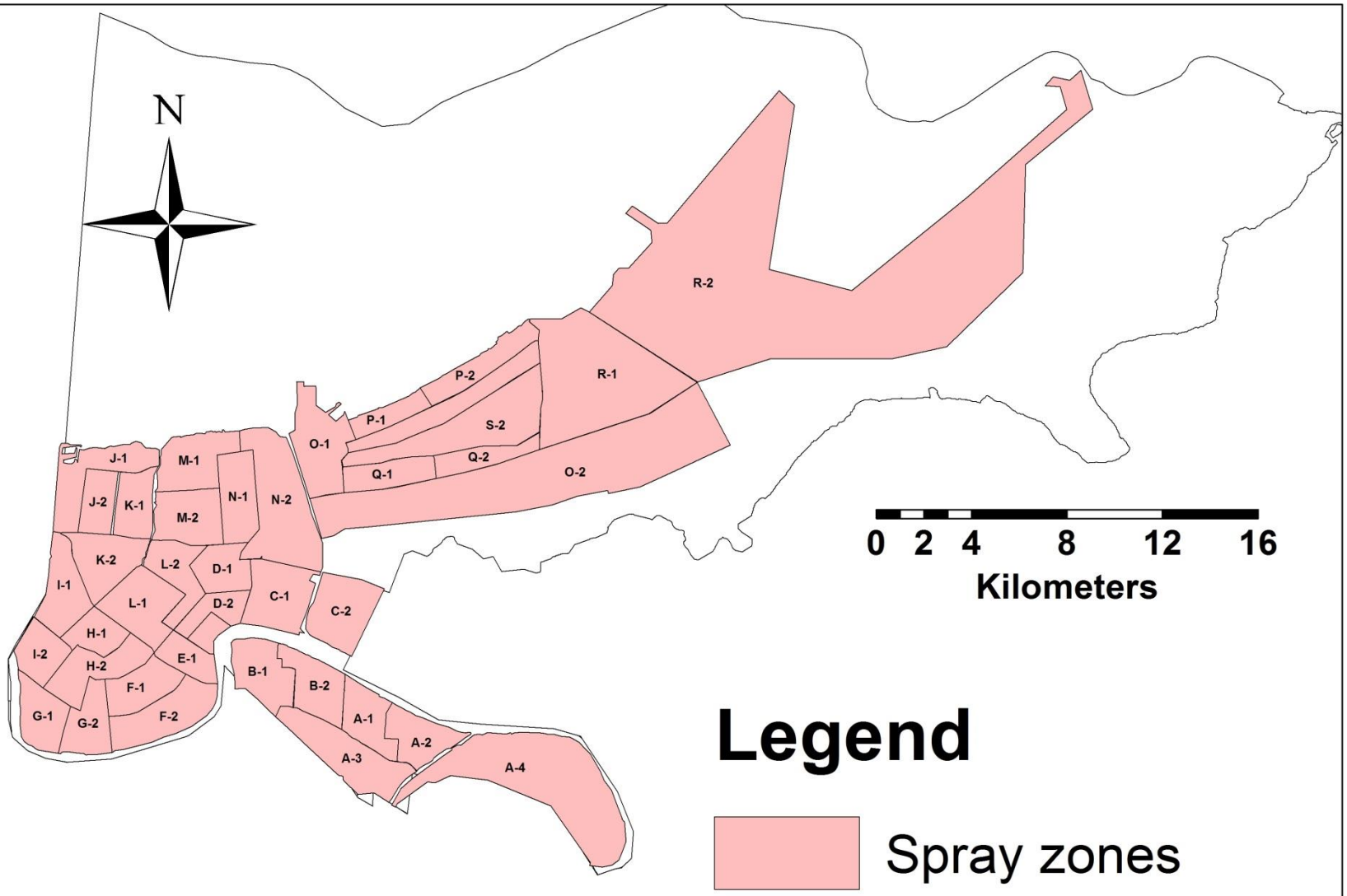
- Common detoxification pathway.
- Target-site insensitivity.

**Multiple-resistance**

- Extends to a variety of insecticides with differing modes of action and different detoxification pathways.

# Cross-Resistance Relationships

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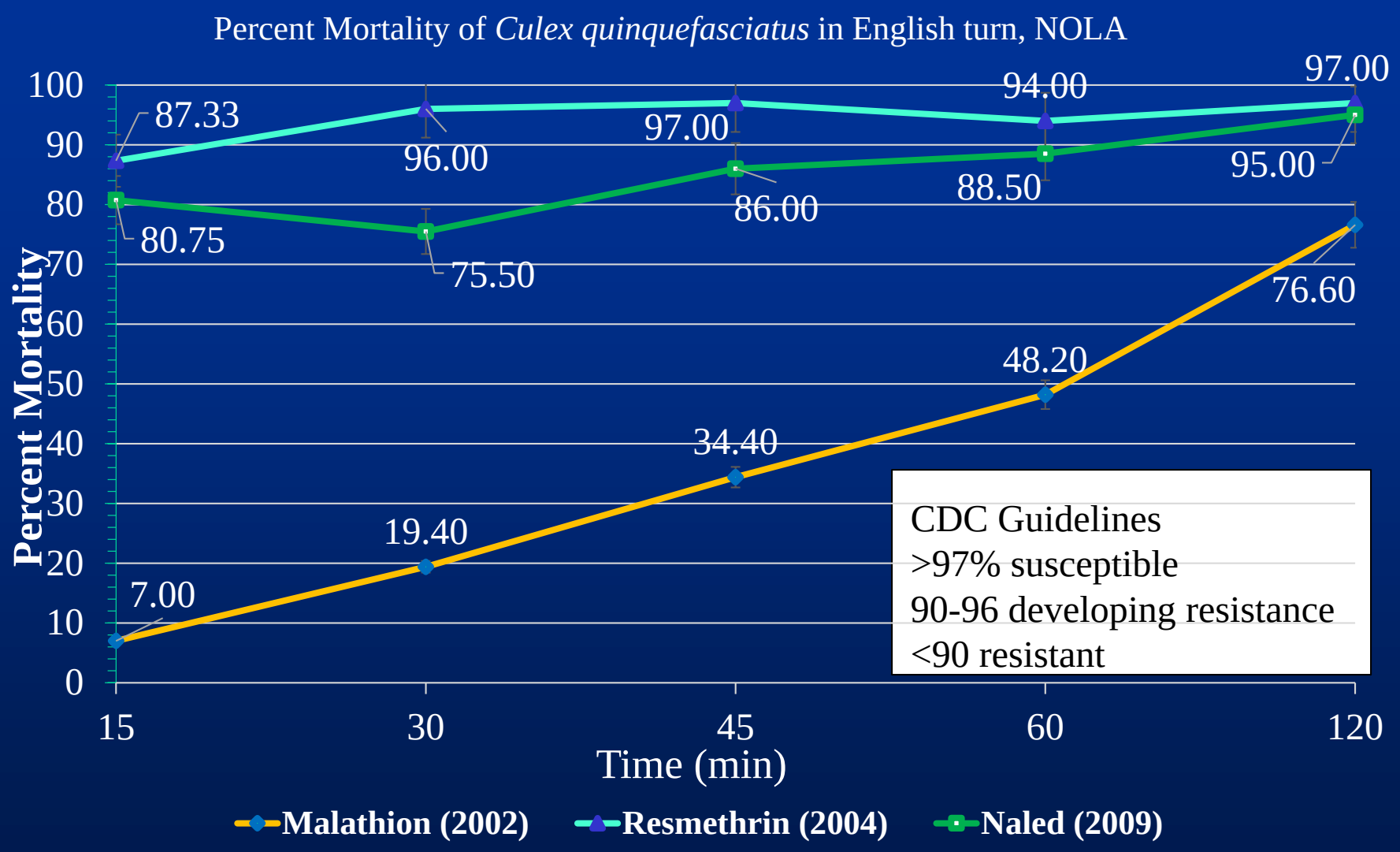
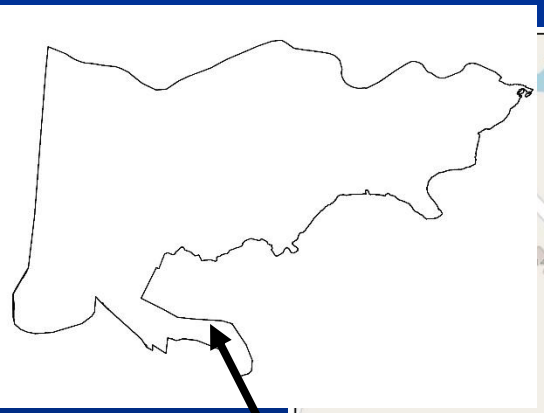
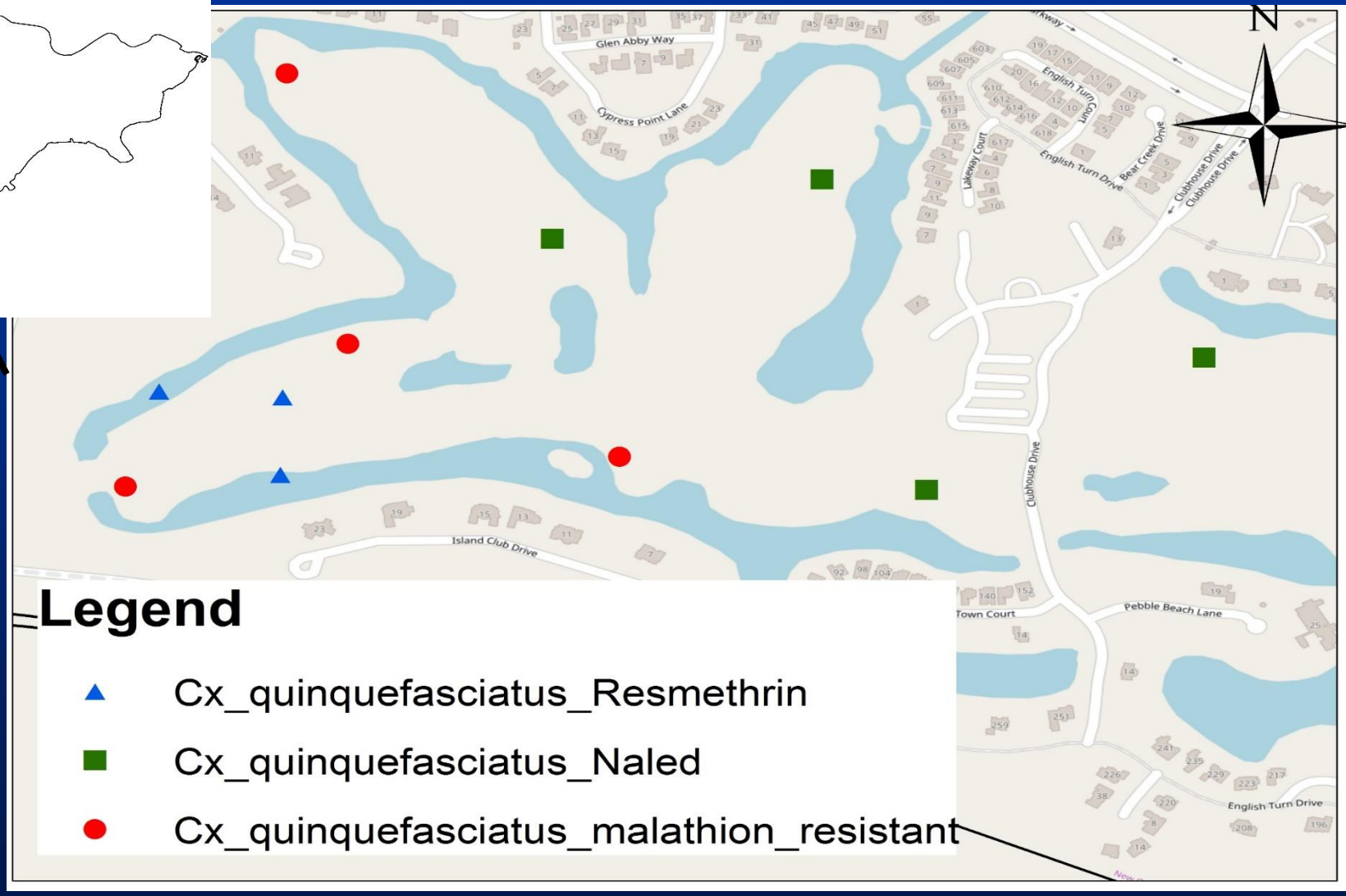
Kilometers
Legend
Spray zones

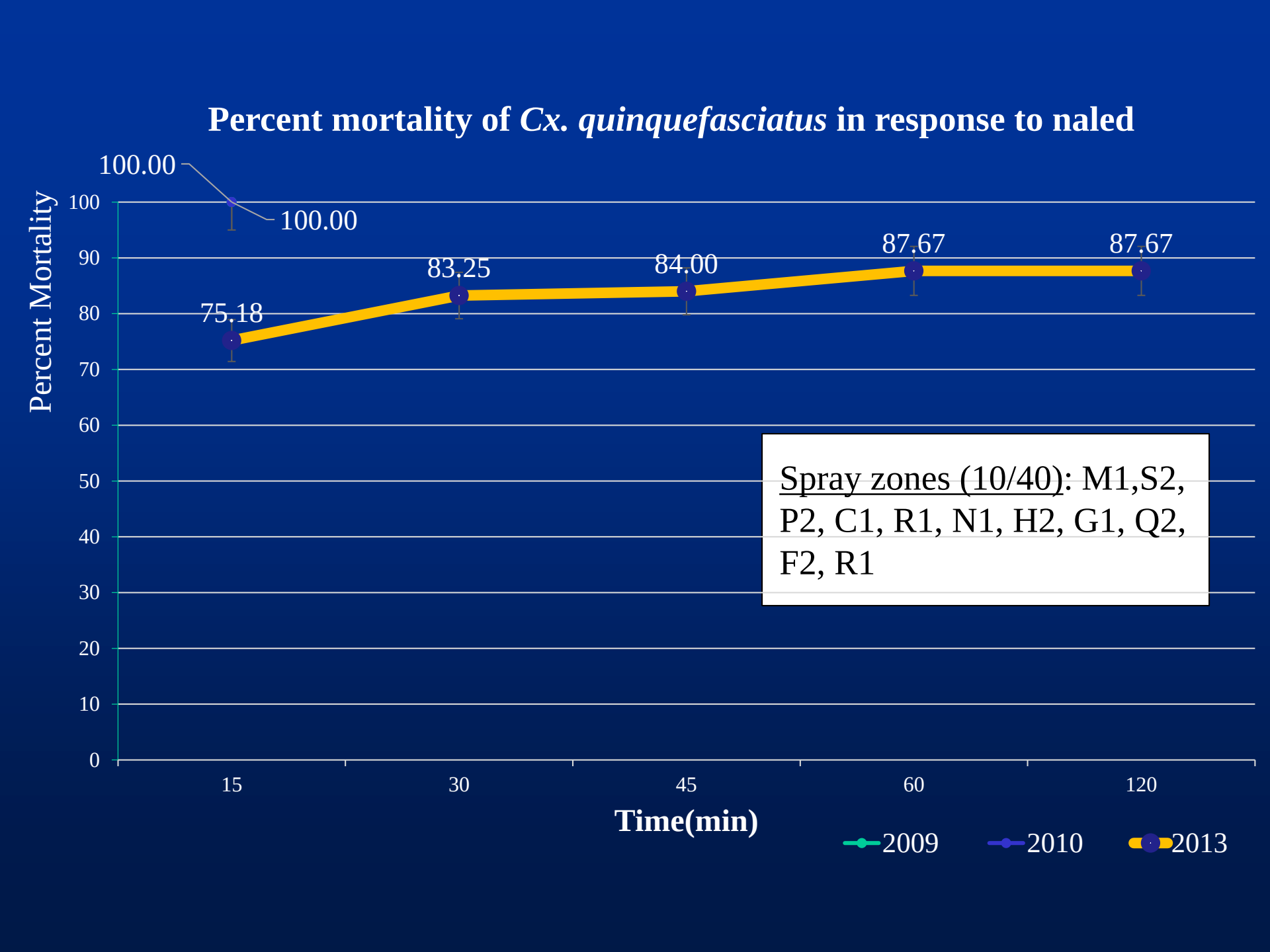
40 zones


Percent Mortality of *Culex quinquefasciatus* in English turn, NOLA
Percent Mortality
Time (min)
100
90
80
70
60
50
40
30
20
10
0
15
30
45
60
120
87.33
96.00
97.00
94.00
97.00
80.75
75.50
86.00
88.50
95.00
7.00
19.40
34.40
48.20
76.60
CDC Guidelines
>97% susceptible
90-96 developing resistance
<90 resistant
Malathion (2002)
Resmethrin (2004)
Naled (2009)

# 2002, 2004, 2009






Percent mortality of Cx. quinquefasciatus in response to naled
Percent Mortality
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100.00
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84.00
87.67
87.67
15
30
45
60
120
Time(min)
Spray zones (10/40): M1,S2, P2, C1, R1, N1, H2, G1, Q2, F2, R1
2009
2010
2013

| *Culex quinquefasciatus* | | | | | |
|---|---|---|---|---|---|
| **Naled spray zones** | **% Mortality/min** | | | | |
| | **15** | **30** | **45** | **60** | **120** |
| **A-4 2009** | 100 | | | | |
| **C-1 2013** | 43 | 100 | | | |
| **F-1 2009** | 100 | | | | |
| **F-2 2013** | 86 | 100 | | | |
| **G-1 2009** | 100 | | | | |
| **G-1 2013** | 100 | | | | |
| **H-2 2013** | 44 | 52 | 100 | | |
| **K-1 2009** | 100 | | | | |
| **K-1 2010** | 100 | | | | |
| **S-2 2013** | 96 | 96 | 100 | | |
| **N-1 2013** | 97 | 97 | 100 | | |
| **O-2 2010** | 100 | | | | |
| **P-2 2013** | 100 | | | | |
| **Q-2 2013** | 64 | 89 | 94 | 100 | |
| **R-1 2009** | 100 | | | | |
| **R-1 2010** | 100 | | | | |
| **R-1 2013** | 75 | 81 | 95 | 100 | |
| **M-1 2013** | 22 | 51 | 63 | 63 | 84 |

| *Culex quinquefasciatus* | | | | | |
|---|---|---|---|---|---|
| **naled trt zones** | **% Mortality/min** | | | | |
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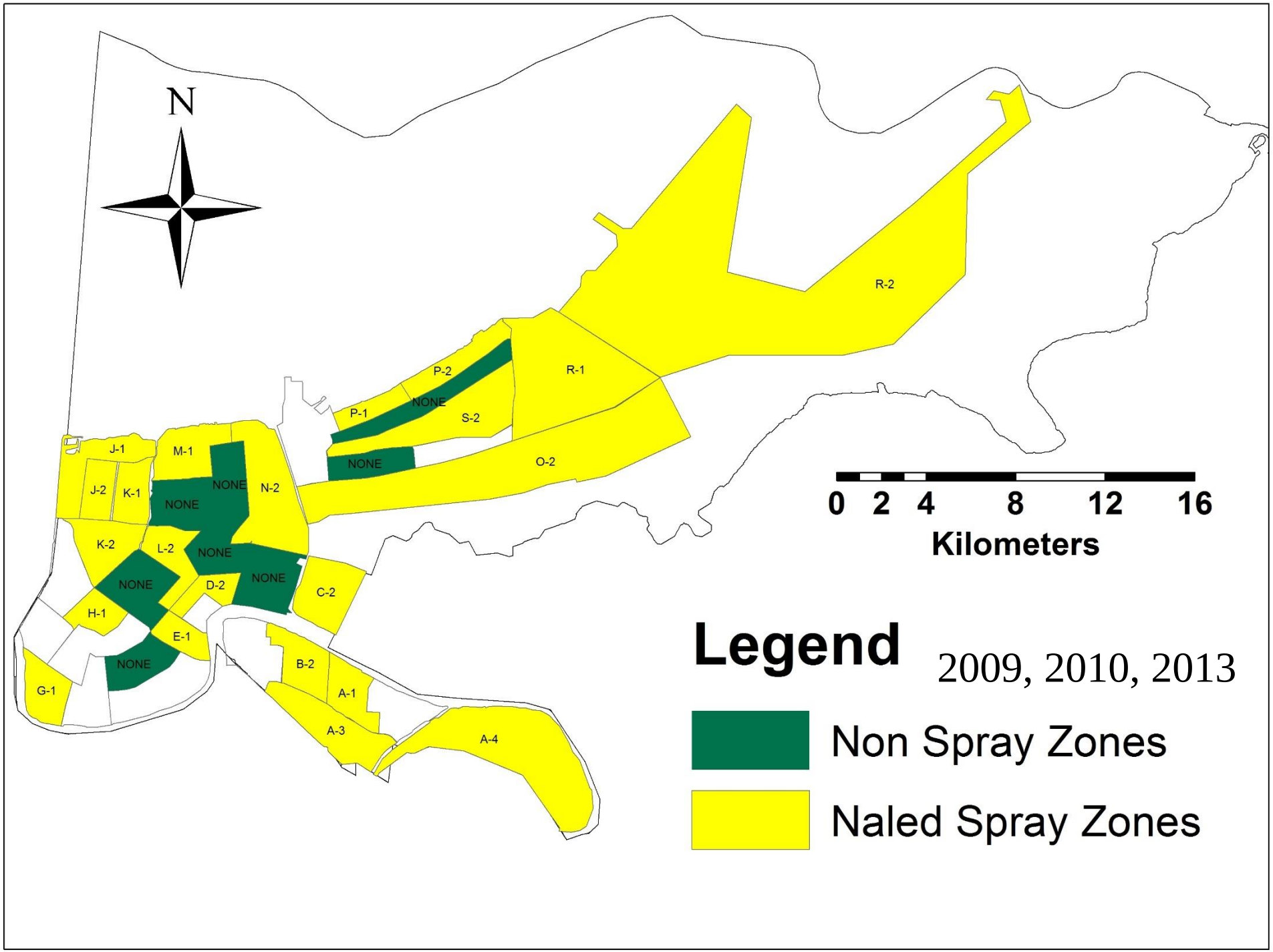
- money
- manpower


N
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Non Spray Zones
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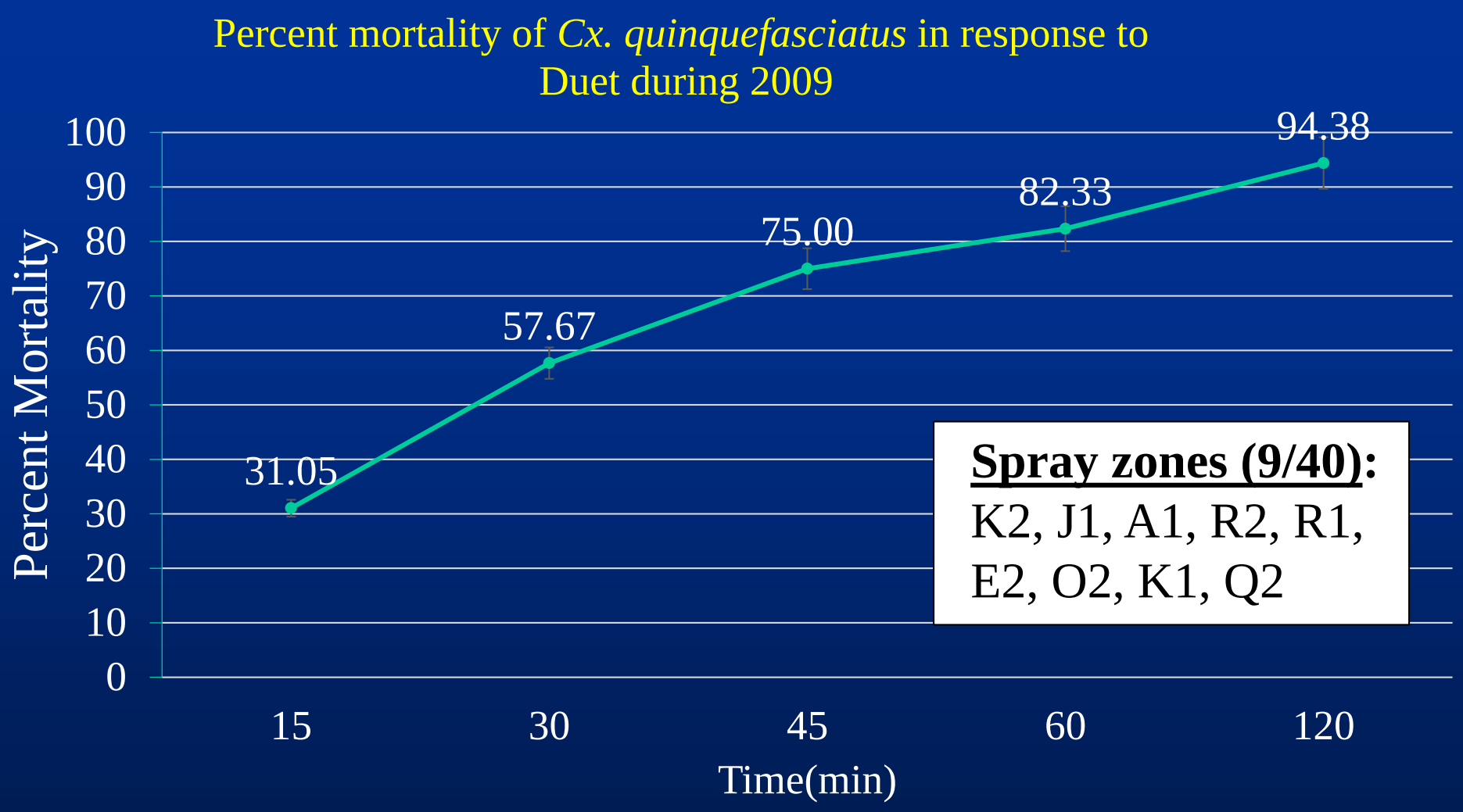
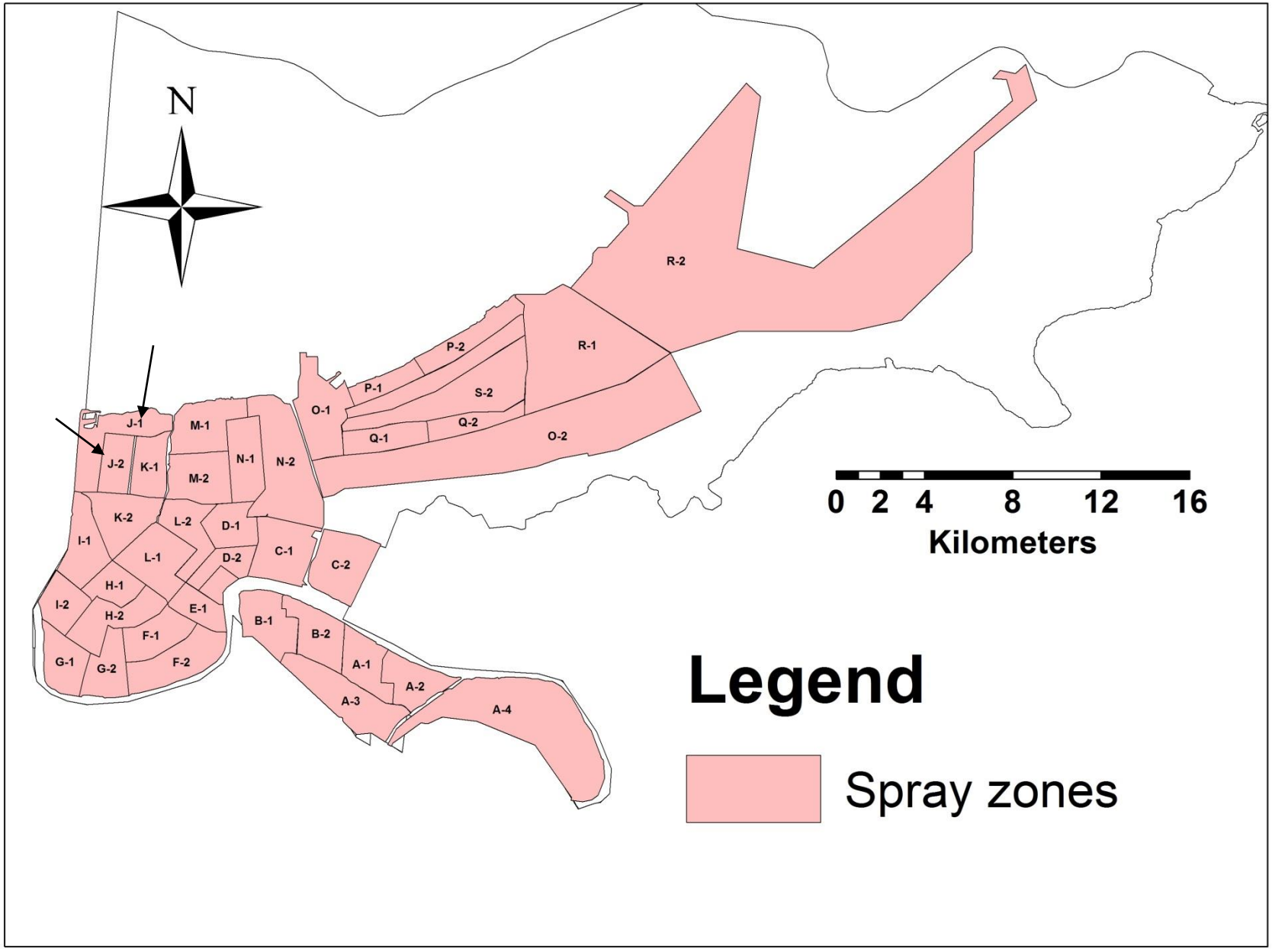



sumithrin and prallethrin

| *Culex quinquefasciatus* | | | | | |
|---|---|---|---|---|---|
| **Duet spray zones** | % Mortality/min | | | | |
| | **15** | **30** | **45** | **60** | **120** |
| **A-1 2009** | 26 | 50 | 70 | 80 | 98 |
| **E-2 2009** | 31 | 79 | 95 | 100 | |
| **J-1 2009** | 30 | 71 | 93 | 97 | 100 |
| **O-2 2009** | 0 | 25 | 66 | 73 | 100 |
| **K-2 2009** | 96 | 99 | 99 | 100 | |
| **R-1 2009** | 57 | 91 | 94 | 97 | 100 |
| **R-2 2009** | 45 | 74 | 90 | 90 | 100 |
| **K-1 2009** | 10 | 34 | 49 | 56 | 95 |
| **A-4 2009** | 29 | 51 | 70 | 79 | 91 |
| **Q-2 2009** | 0 | 36 | 52 | 70 | 86 |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E-1
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G-1
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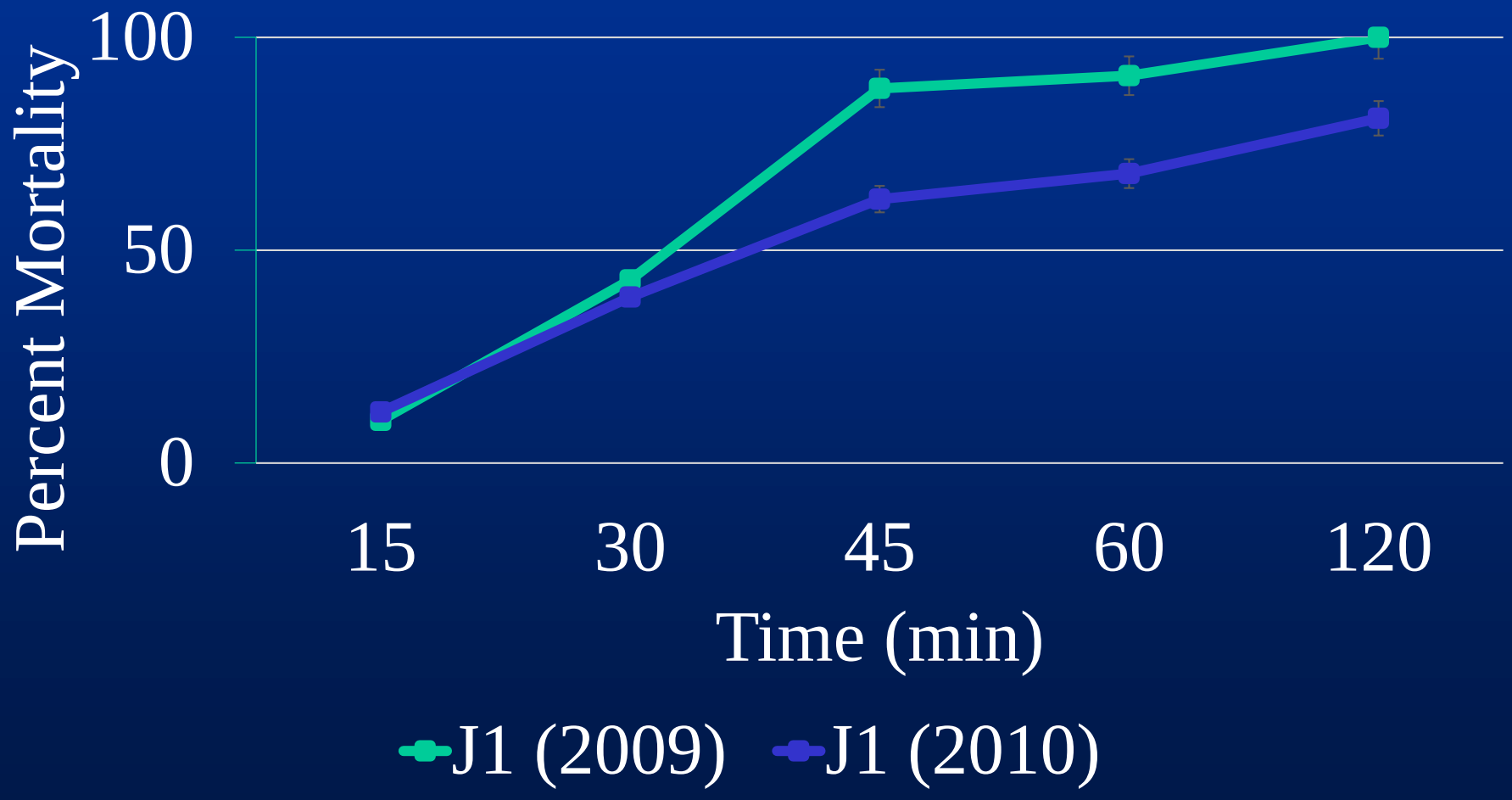
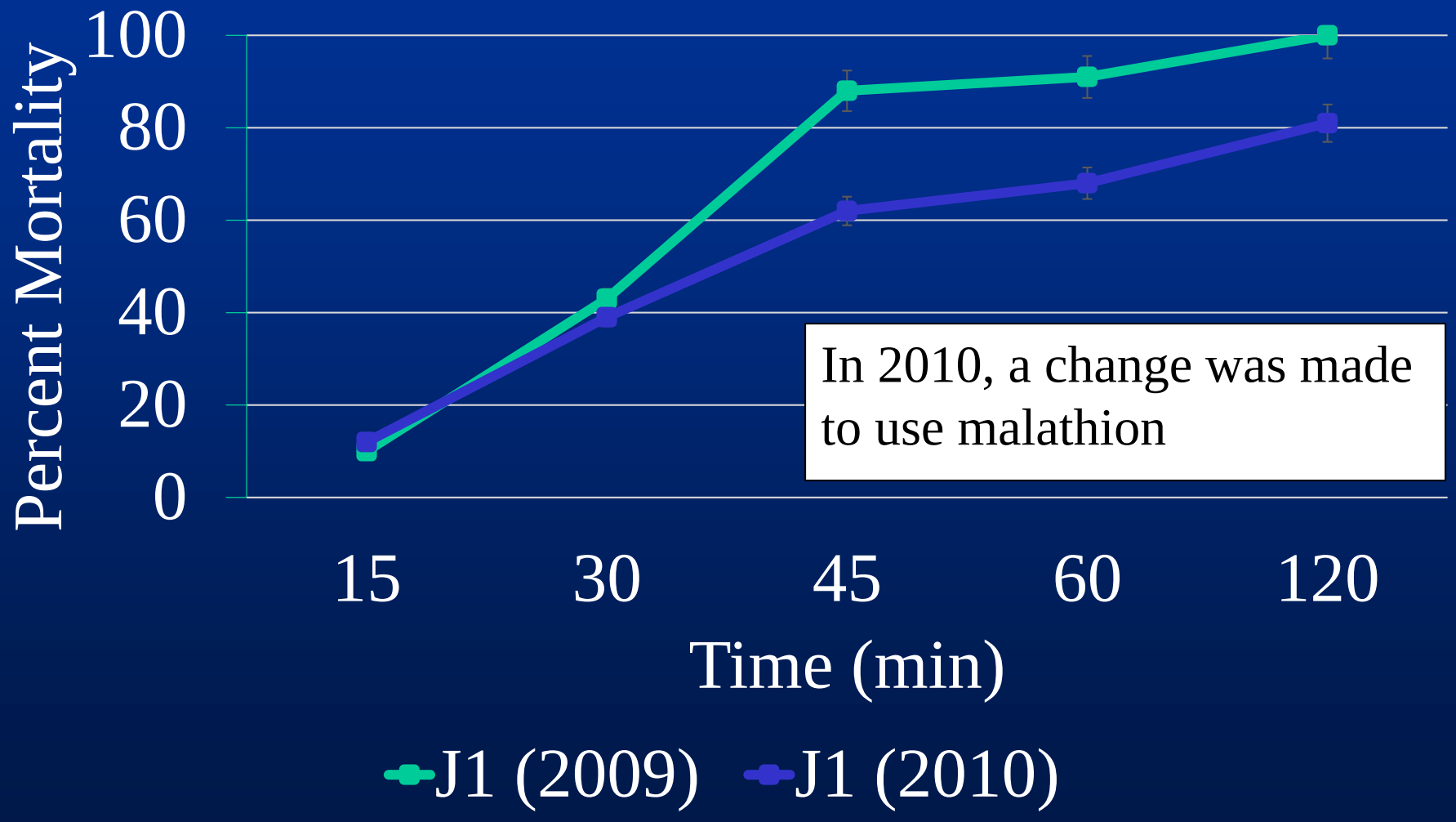
F-2
A-1
A-2
A-3
A-4
0 2 4 8 12 16
Kilometers
Legend
Spray zones

# Developed resistance in *Cx. quinquefasciatus* to resmethrin



# Developed resistance in *Cx. Quinquefasciatus* to resmethrin



# Lesson Learned

- Data mining
  - Keep consistent data records
- Multiple levels of resistance within a neighborhood
- Select consistent sampling sites
- Real-time evaluation of field mosquito populations correlated with pre- and post treatment season

# Strategies for Preventing Resistance

- Reduce your dependence on insecticides.
- Use materials with differing modes of action for different life stages.
- Alternate classes of insecticides.
- Treat only when absolutely necessary.
- Monitor both your control efficacy and resistance development.

# Strategies implemented in NOLA

# Field Procedure

- Cages were placed in open and sequestered locations at multiple points in each zone
  - Three sites per zone were paired with spinners
- Pre-treatment mortality was recorded prior to placement
- Aerial treatment lasted for ~45 minutes (8:02 pm – 8:47 pm)
  - Application rate: 0.5 oz / acre (50% of the maximum labeled rate)
- Cages and spinners were left for 1 hour post-treatment


# Mortality Results

## *Aedes aegypti*



**<u>Zone A</u>**

**Average mortality AAEG Open:** 90.71%

**Average mortality AAEG Sequestered:** 90.18%

**<u>Zone B</u>**

**Average mortality AAEG Open:** 90.18%

**Average mortality AAEG Sequestered:** 81.66%

Field validate methodology

# Mortality Results

## *Aedes albopictus*



**<u>Zone A</u>**

**Average mortality AALB Open:** 99.62%

**Average mortality AALB Sequestered:** 91.97%

**<u>Zone B</u>**

**Average mortality AALB Open:** 73.57%

**Average mortality AALB Sequestered:** 66.36%

# Larvicides

# Area-wide Larviciding

- Similar to adulticide technology – wind-driven droplet deposition
- Larger droplet size (100 to 300 µm) light enough to stay aloft temporarily, but heavy enough to settle into containers
- Evaluated in suburban habits – NJ & FL
- Used in Miami, FL following local transmission Zika virus
- Variety of chemical and equipment combinations in use
- Expensive but effective – epidemic vs routine
- Purchased equipment, made modifications to optimize deposition and efficiency

# Methods

- March 2017 - field trials with a modified Buffalo Turbine (BT) and Curtis Dyna-Fog Ag-Mister LV8™ Agriculture Low Volume Sprayer (LV8)
- VectoBac® WDG (Valent Biosciences)

- **Open field trial -** Eastern New Orleans, single pass application from each machine, bioassay jars and kromekote cards were set in a grid of 5 rows at 50' intervals from the application site (0-600'), 100' (700-900')

- **Neighborhood trial -** Holy Cross neighborhood of the Lower 9th Ward, 2 "runs" for each machine; a single pass and double pass treatment method, utilizing bioassay jars only at 50' intervals in yards in open and sequestered locations

# Methods

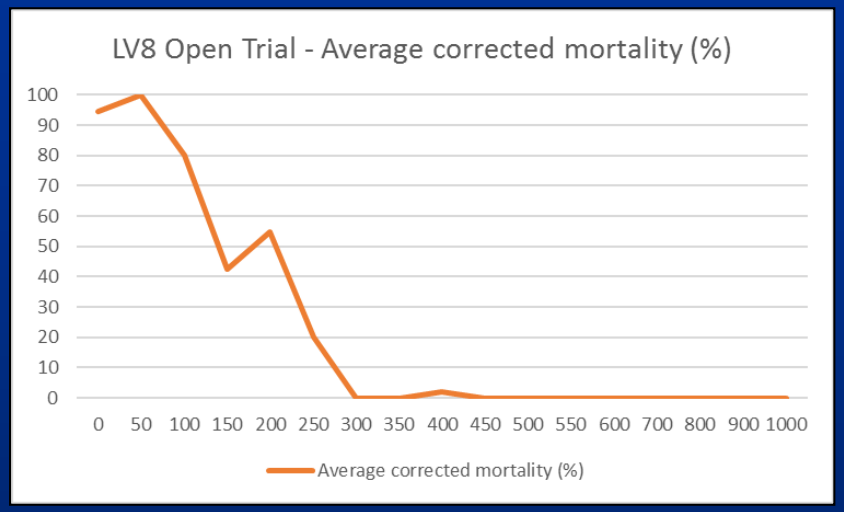
- VectoBac® WDG mix rate 1 lb per gallon of water, red dye added for droplet visualization
- Bioassay jars and kromekote cards left in the field 30 minutes post-application to allow for deposition
- At the Biological Control lab, 200ml of water and 15 – 1st instar laboratory-reared *Ae. aegypti* added to each bioassay jar
- Larvae were monitored at 1 hour, 24 hours, and 48 hours for mortality
- Kromekote cards were scanned utilizing ImageJ software (NIH), R statistical software and a macro script
- Droplet size and density were measured

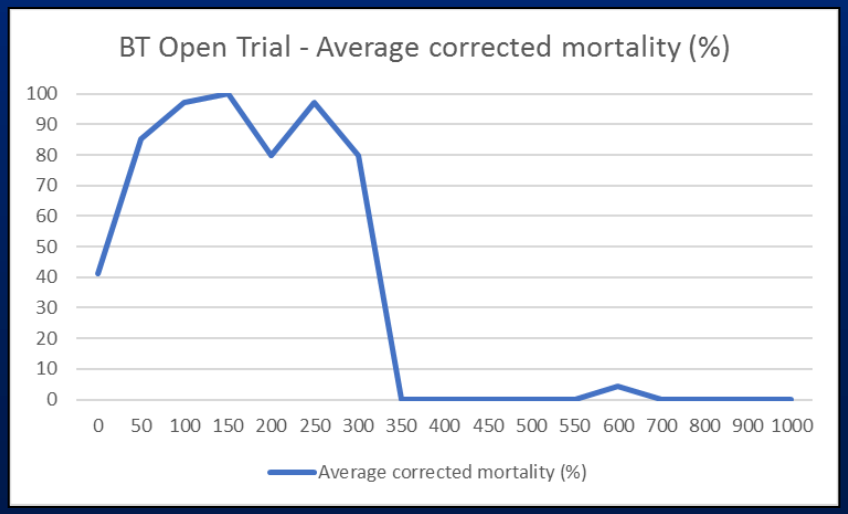
Curtis Dyna-Fog Ag-Mister LV8™ Agriculture Low Volume Sprayer (LV8)

Buffalo Turbine

# Results – Open Field

- Wind speed ranged from 5.8-8.1 mph WNW
- **LV8** - peak average mortality (94.7-100%) 0-50ft, mortality observed up to 250ft, but almost no larval mortality past 300 ft
- **BT** - from 0-50 ft lower average mortality (41.3%), but higher 50-300ft (80-100%), almost no larval mortality was observed past 300ft
- Average droplet counts supported mortality observations

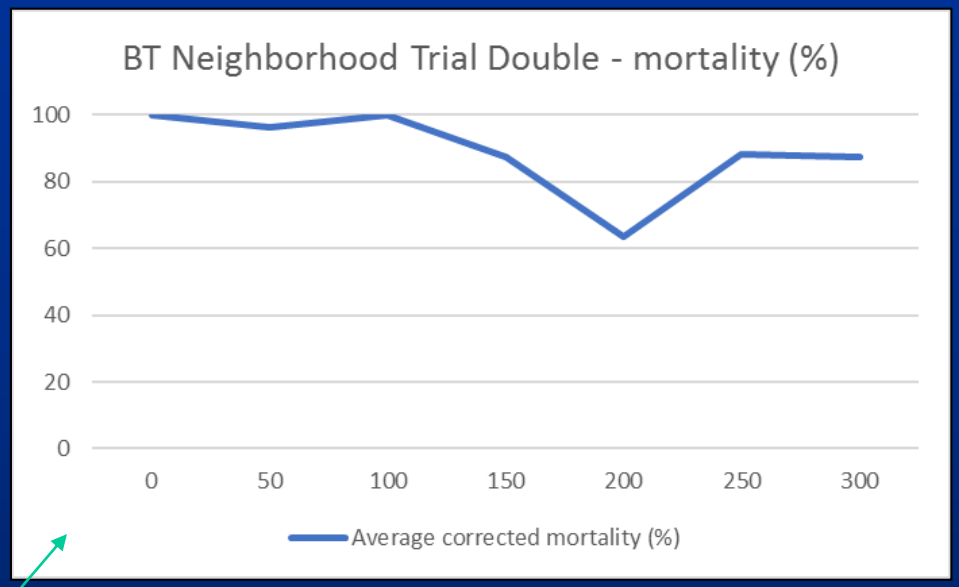


# Trial 2 - Neighborhood


**Single pass application**


**Double pass application**




| Distance (in ft) | Average corrected mortality (%) |
|---|---|
| Front Open | 100.0 |
| Front Sequestered | 89.5 |
| Back Open | 82.3 |
| Back Sequestered | 65.5 |

# Physical Control = Source Reduction

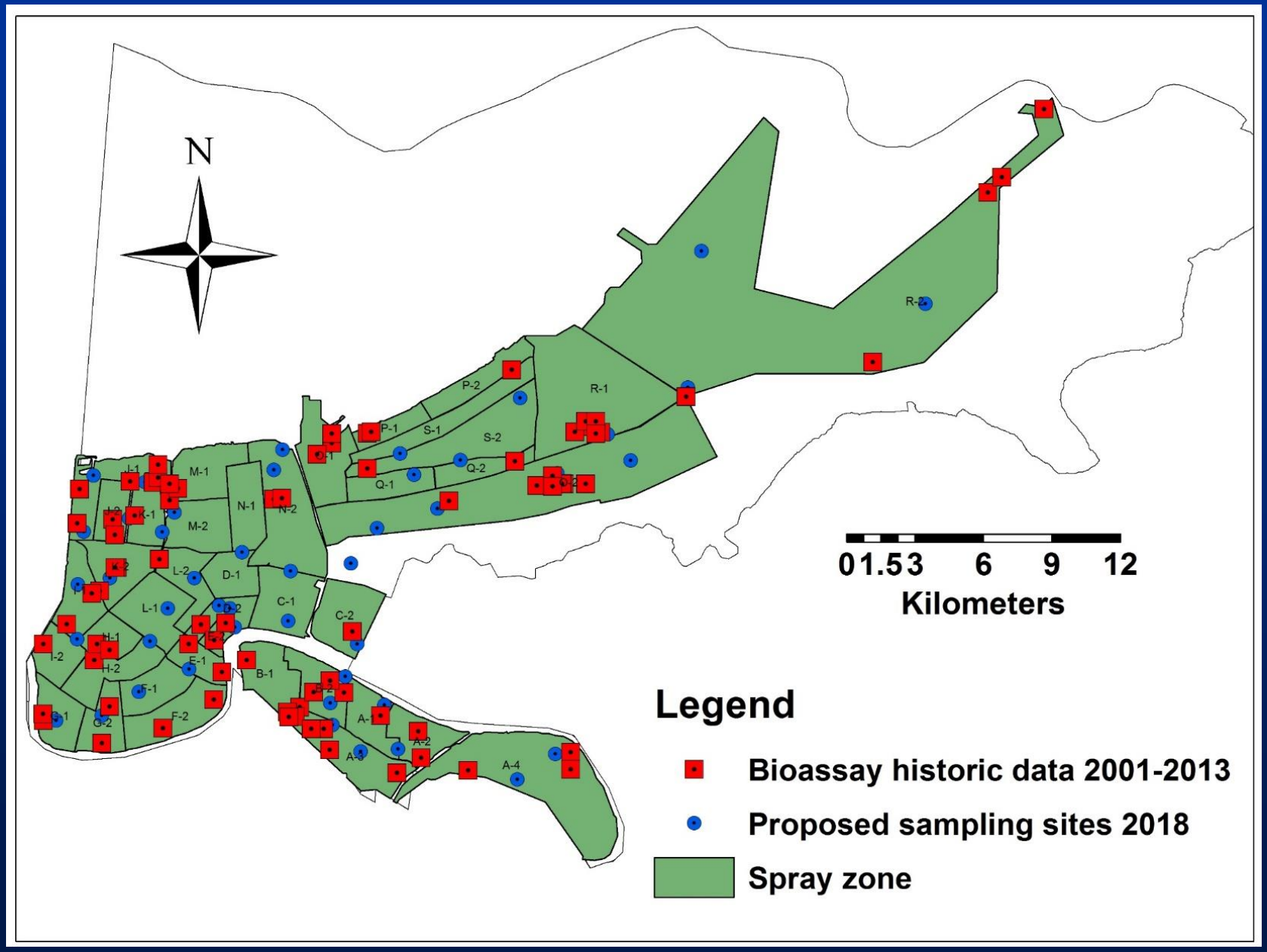
- Physical alteration of mosquito breeding sites to prevent mosquito from completing their life cycle
- NOT permanent control

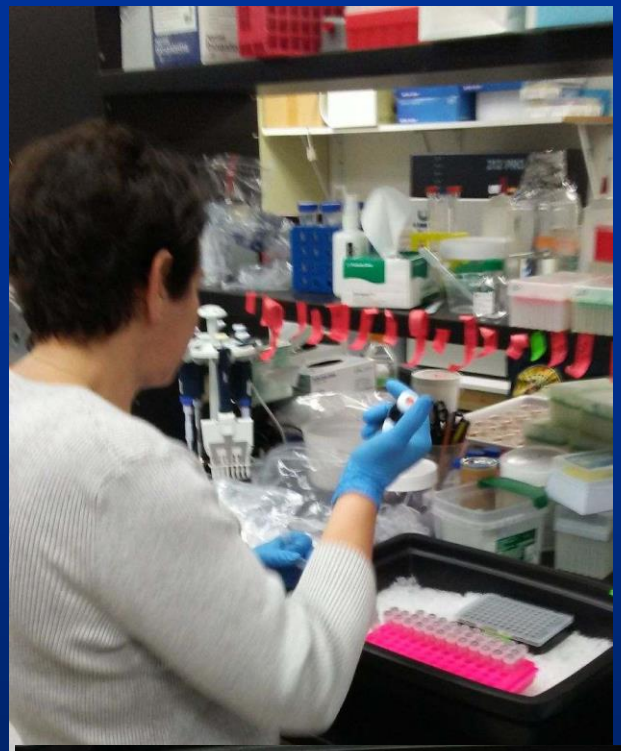
# Biological Control

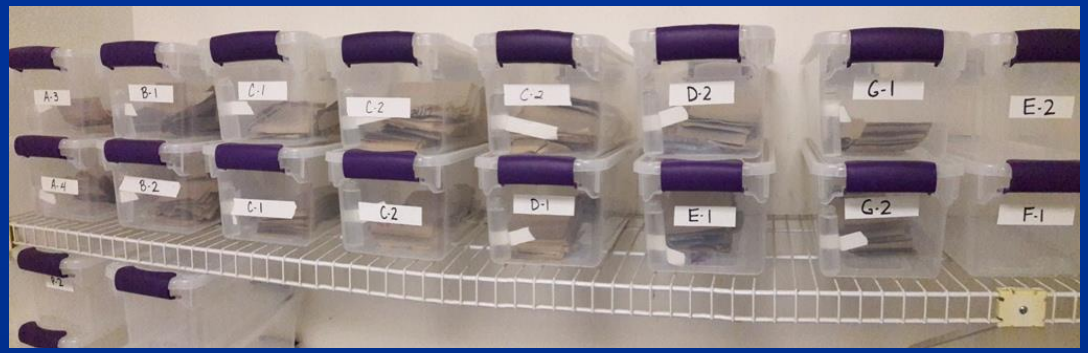
Copepods

Fish

# Current project (2017-2018)

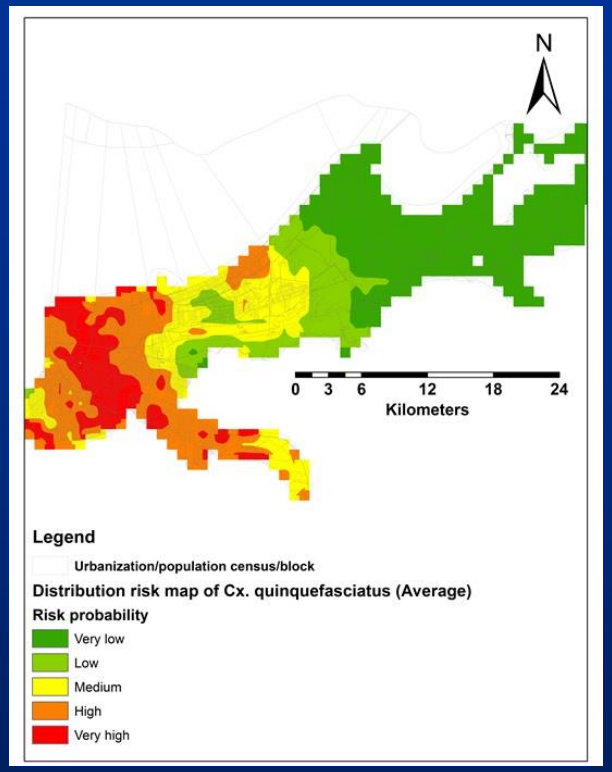


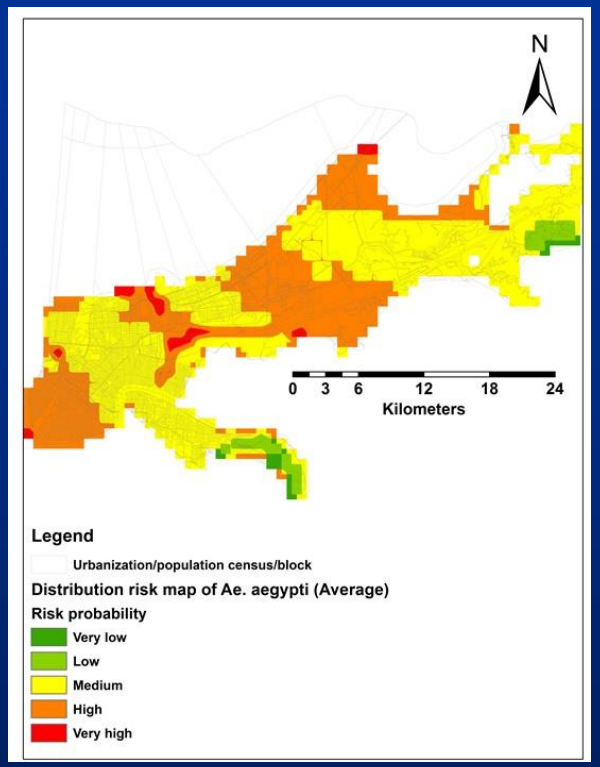



A-3
B-1
C-1
C-2
C-2
D-2
G-1
E-2
A-4
B-2
C-1
C-2
D-1
E-1
G-2
F-1

# GIS: Data Output

(Mapping and Prediction of Vector/Disease Distribution)

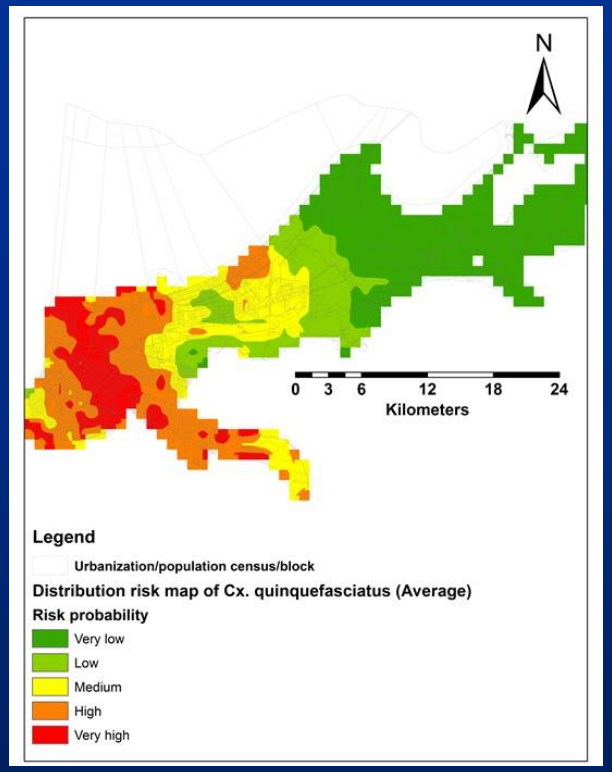


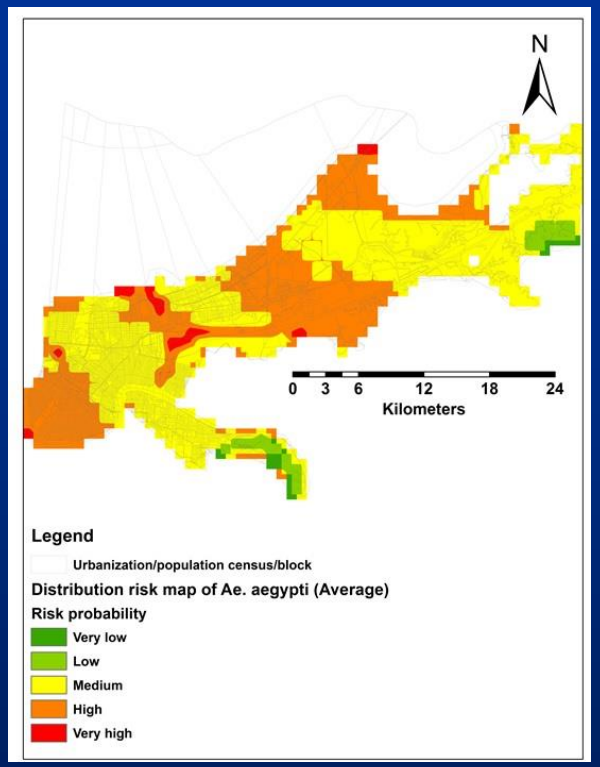




- Can we:
  - model resistance?
  - Create risk maps?

# GIS: Data Output

(Mapping and Prediction of Vector/Disease Distribution)




You bet!

- Can we:
  - model resistance?
  - Create risk maps?

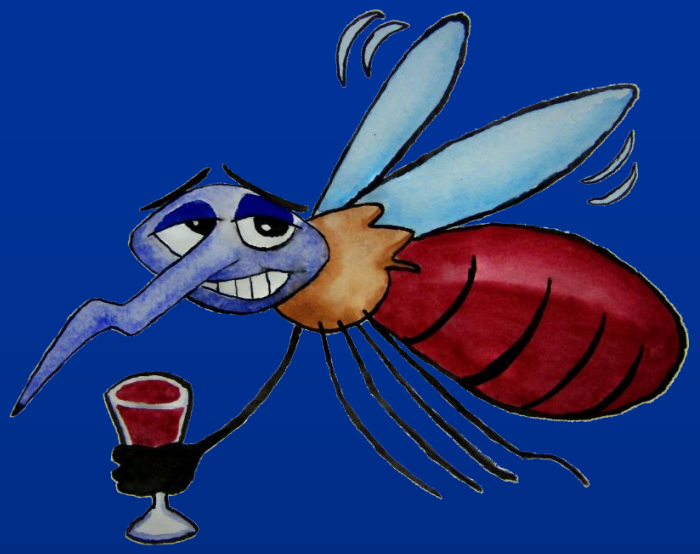
We just need the data

CDC

# Preparedness

- A response plan
- Robust surveillance program
  - Mosquito collections
  - Virus testing
  - Understanding of local mosquito resistance/tolerance to insecticides
  - Data management
- Infrastructure
  - Equipment (larviciding and adulticiding)
  - Supplies and insecticides (validated in the field)
  - Trained personnel
  - Contingency contracts
- Interagency cooperation
- Education
  - Government officials, medical professionals, port and airport officials, public

# Thank you



NOMTCB employees
CDC
- Dr. McAllister
- A. Ruiz
- Technical support and financial support

LA Dept of Infectious Disease – EPI group
Valent BioSciences
AMVAC
Manatee County MAD
Dr. P. Koehler and Dr. R. Pereira (UFL)

Teamwork is the key to success.

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