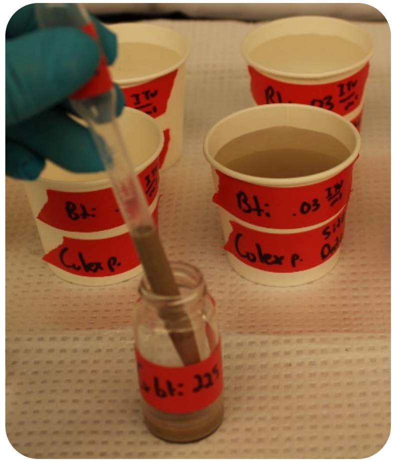
Insecticide Resistance in Mosquitoes:
Practical Guidance and Tips for Performing Your Own Monitoring Assays



Presented by:

James Burtis, Cornell University

John Shepard, Connecticut Agricultural Experiment Station

1 April 2020

Monitoring Pesticide Resistance in Disease Vectors

James Burtis

Postdoctoral Associate

Cornell University

Important Points

- What is pesticide resistance and how does it emerge?
- How are resistance bioassay diagnostics created?
- How do you determine the resistance status of field-collected mosquitoes?
- How can the results of these resistance tests inform management?
- How do you collect mosquitoes quickly for resistance assays?
- How do you rear different mosquito species in the laboratory for resistance assays?

Why is Monitoring Pesticide Resistance Important?

- Many classes and modes of action are available, but few are used for mosquito control
- State regulations can further restrict the ability to cycle through different pesticides
- Resistance monitoring is necessary to maintain the efficacy of the few products that are available for vector control

Additional EPA Information About Pesticides for Mosquito Control

https://www.epa.gov/mosquitocontrol/controlling-mosquitoes-larval-stage

https://www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes

Pesticide Classification

Acaricides
(14 active ingredients)

Botanical Insecticides
(5 active ingredients)

Carbamates
(1 active ingredient)

Chlorinated Hydrocardbon Insecticides
(2 active ingredients)

Formamidines
(1 active ingredient)

Fumigants
(1 active ingredient)

Inorganic Insecticides
(1 active ingredient)

Insect Growth Regulators
(5 active ingredients)

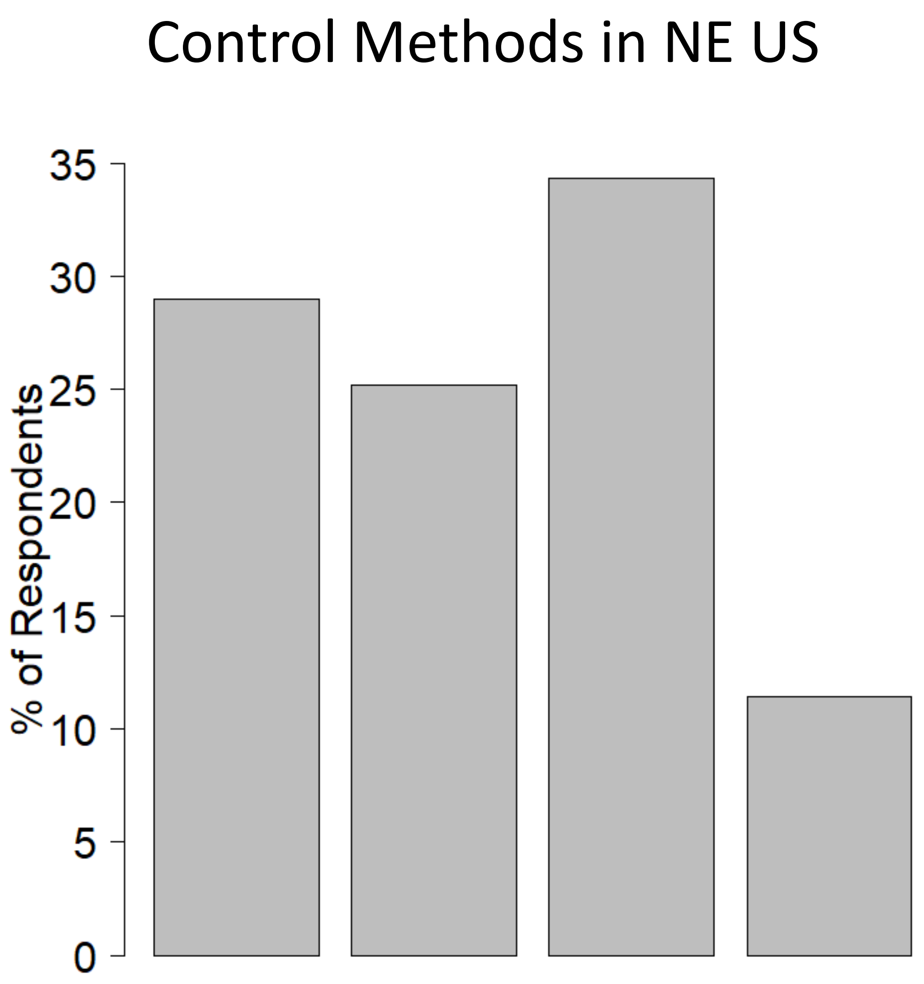
Microbial Insecticides
(3 active ingredients)

Neonicotinoids
(1 active ingredient)

Organophosphates
(6 active ingredients)

Pyrethroids
(1 active ingredient)

Why is Monitoring Pesticide Resistance Important?



Pesticide Classification

Acaricides
(14 active ingredients)

Botanical Insecticides
(5 active ingredients)

Carbamates
(1 active ingredient)

Chlorinated Hydrocardbon Insecticides
(2 active ingredients)

Formamidines
(1 active ingredient)

Fumigants
(1 active ingredient)

Inorganic Insecticides
(1 active ingredient)

Insect Growth Regulators
(5 active ingredients)

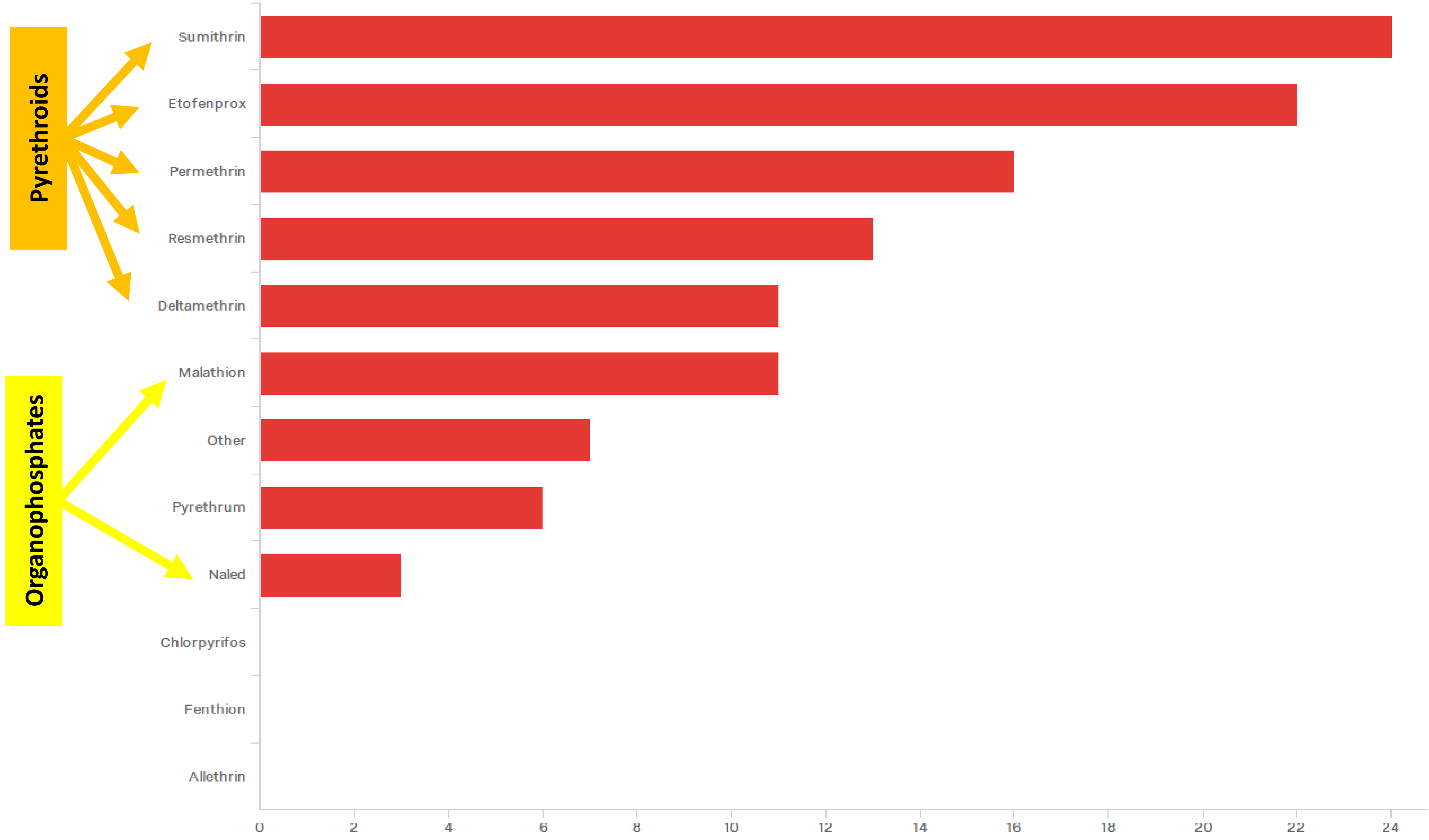
Microbial Insecticides
(3 active ingredients)

Neonicotinoids
(1 active ingredient)

Organophosphates
(6 active ingredients)

Pyrethroids
(1 active ingredient)

Adulticides Used in Our Region



Resistance Mechanisms

	Biochemical mechanism of resistance				
	Metabolic			Target-site	
	Esterases	Monooxygenases	GSH S-Transferases	kdr	Altered AChE
Pyrethroids	•	●		●	
DDT		•	●	●	
Carbamates	•				●
Organophosphates	●	•			●

(Circle size reflects the relative importance of the mechanism on resistance)

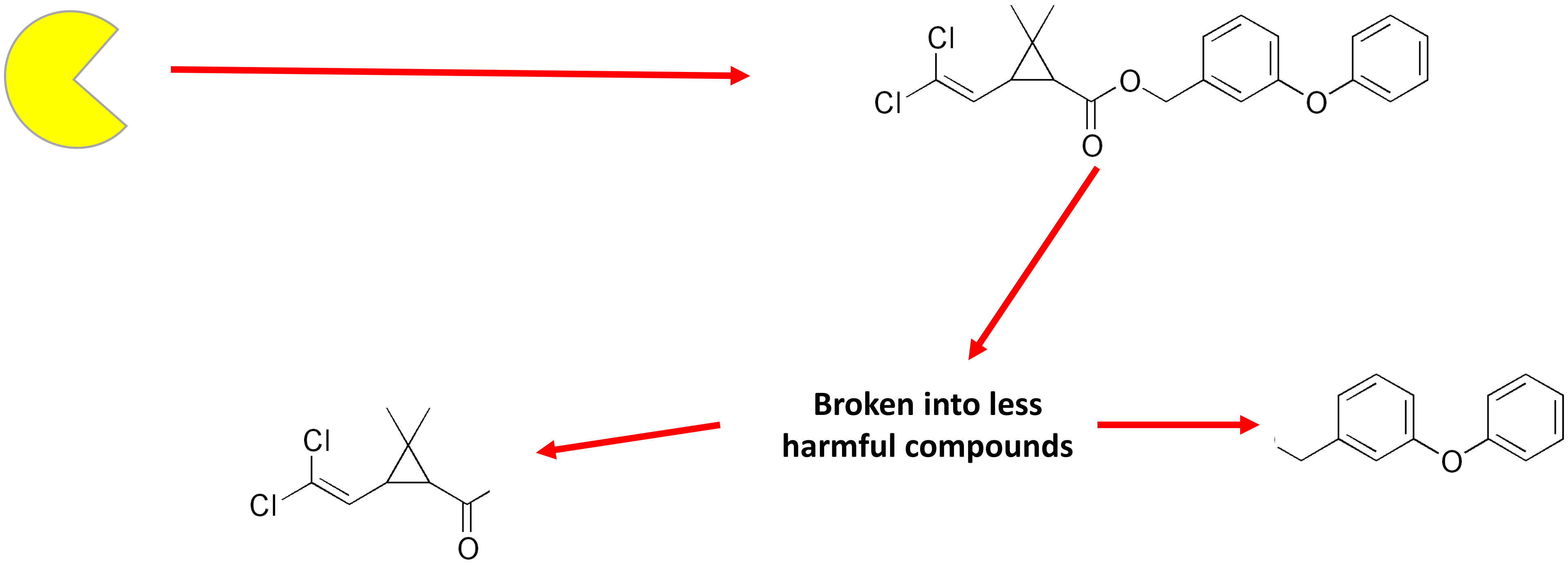
Source: IRAC (2011) Resistance Management for Sustainable Agriculture and Improved Public Health

Resistance Mechanisms

<u>Metabolic Resistance</u>: Components of an insect's metabolic system adapt to break down insecticides (*Esterases / Monooxygenases / Glutathione S-Transferases)*

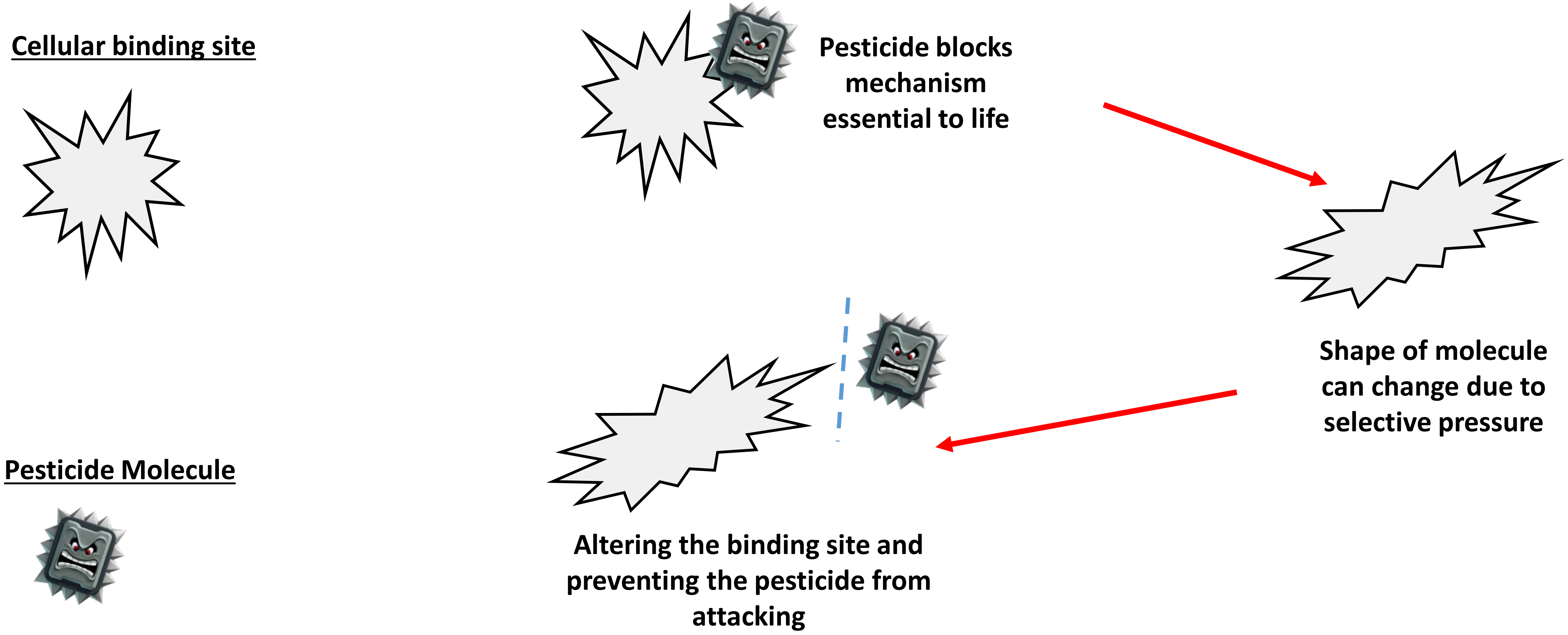
<u>Oxygenase</u>

<u>Permethrin</u>



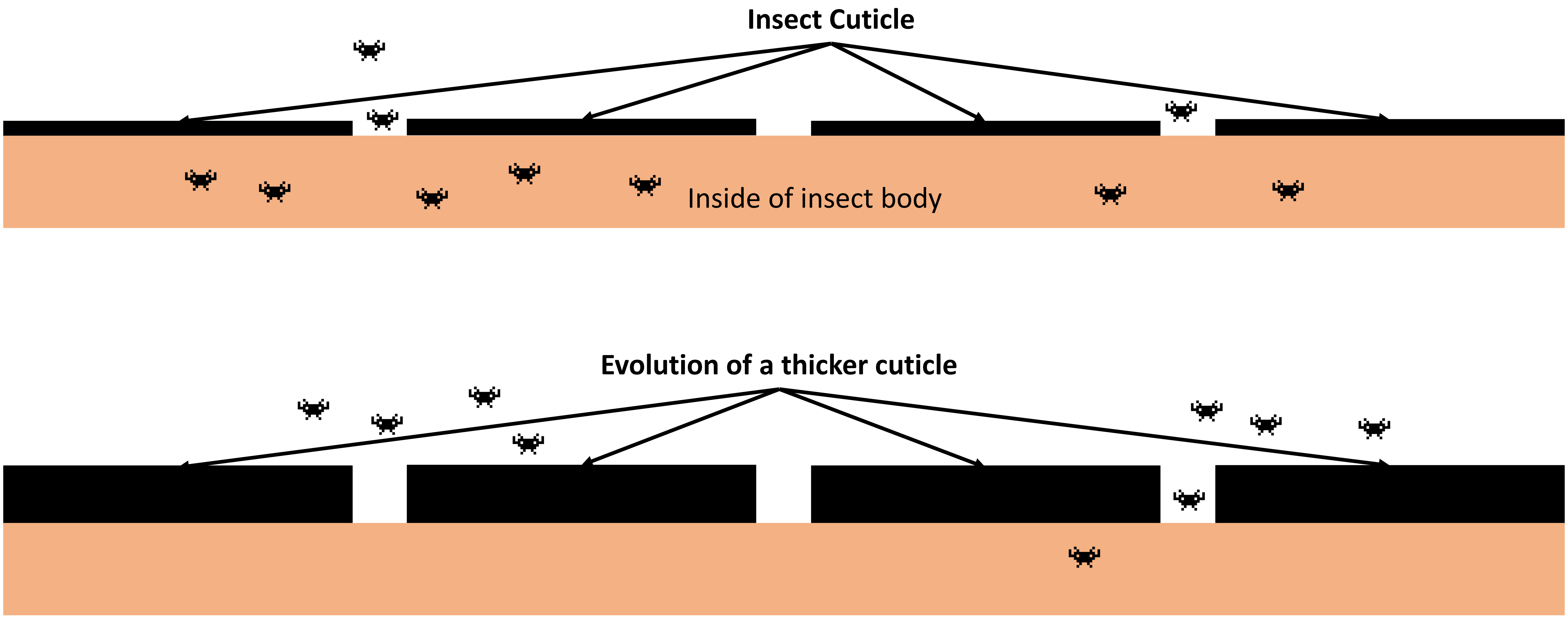
Resistance Mechanisms

<u>Altered Target-Site Resistance</u>: The site where the pesticide binds becomes modified and reduces efficacy *(Knock Down Resistance / Modified Acetylcholinesterase)*

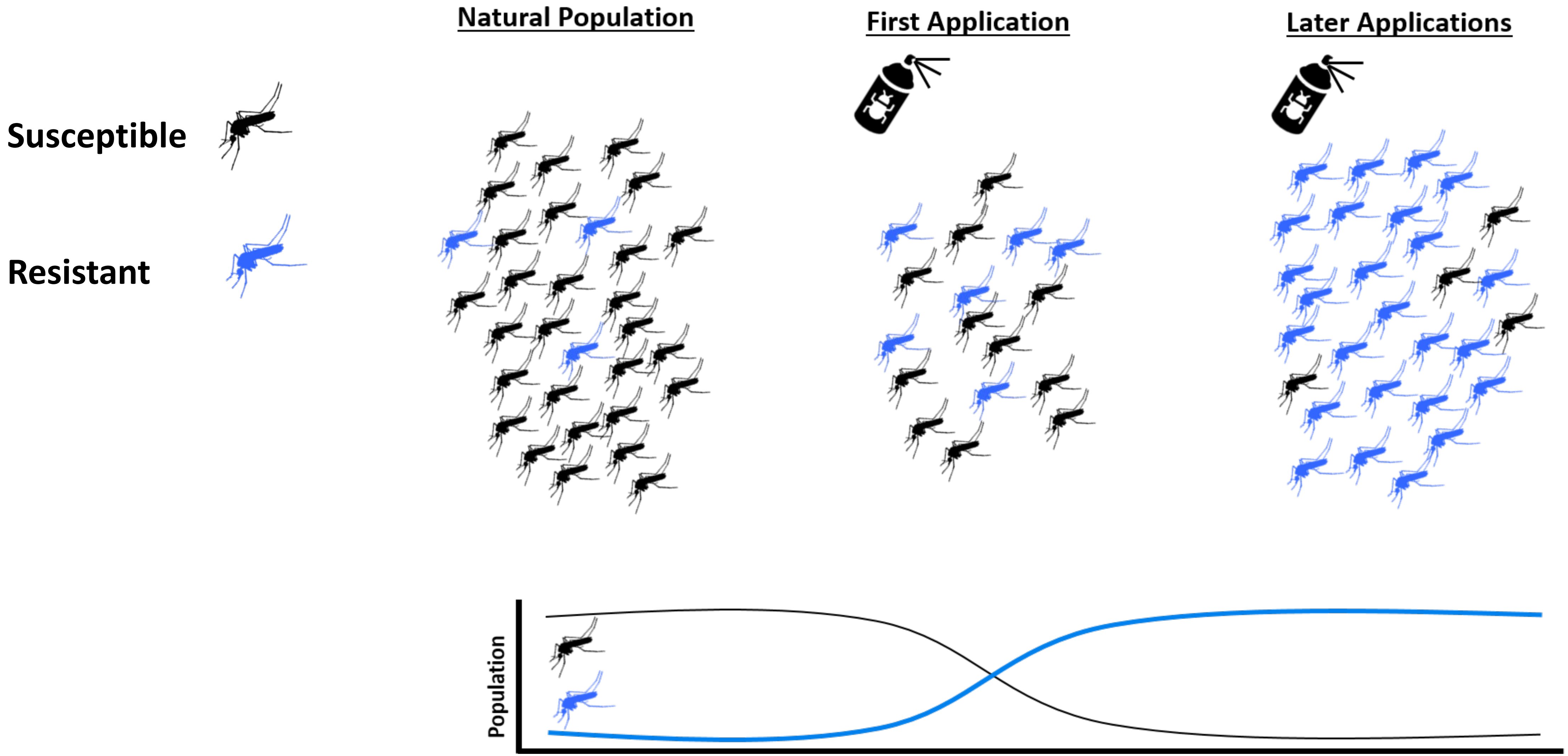


Resistance Mechanisms

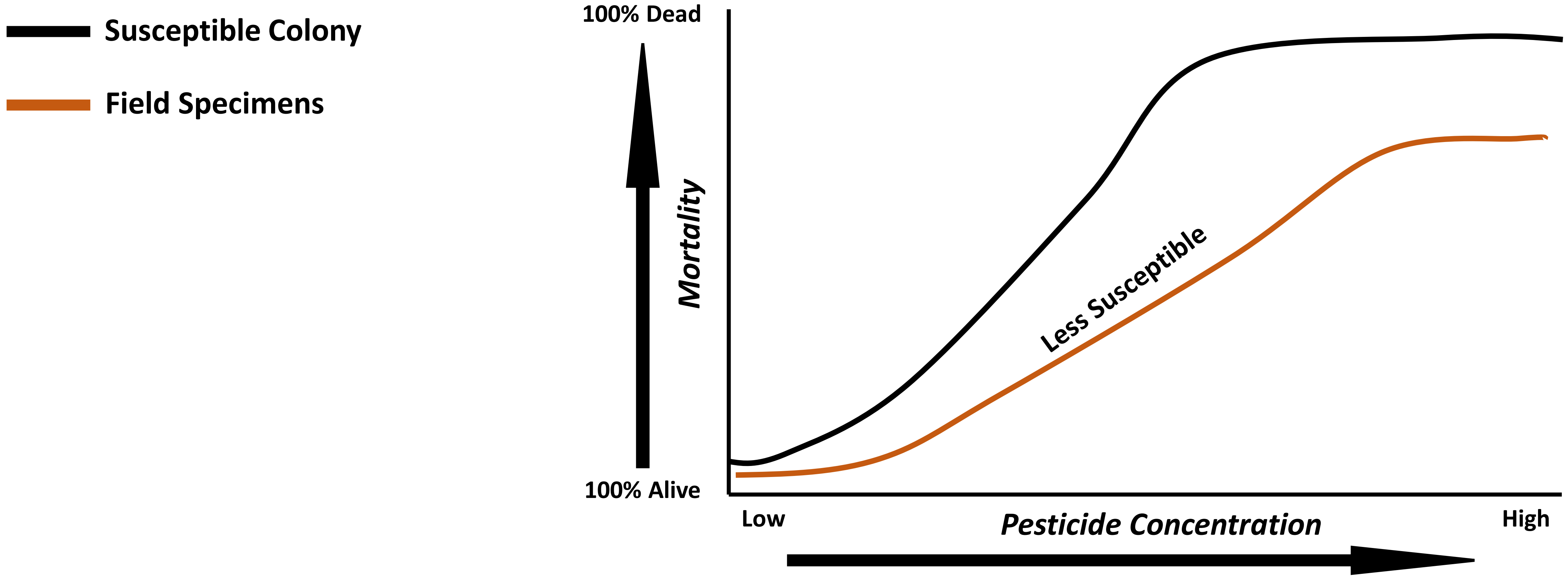
Penetration Resistance: Resistant insects absorb toxins more slowly, which occurs when the insects' outer cuticle develops barriers



How Does Resistance Emerge?

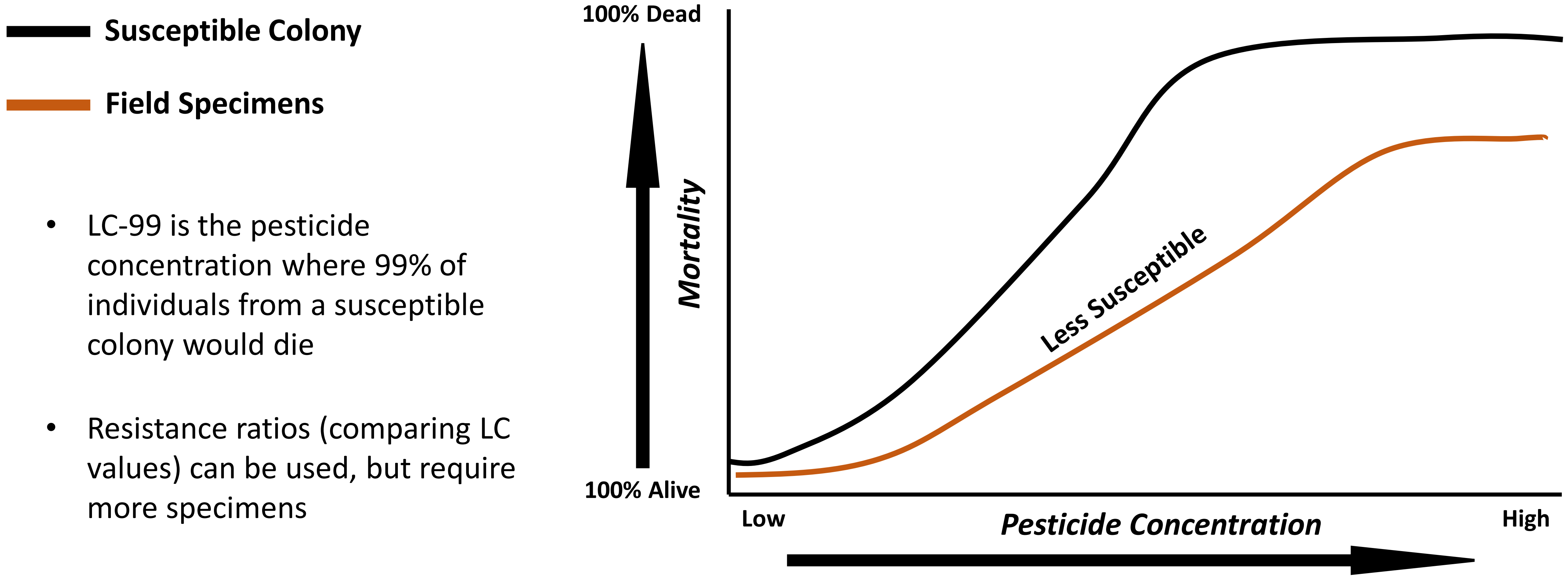


Resistance vs. Susceptibility



Susceptibility is determined by comparing mortality curves of field collected mosquitoes to a susceptible strain

Resistance vs. Susceptibility

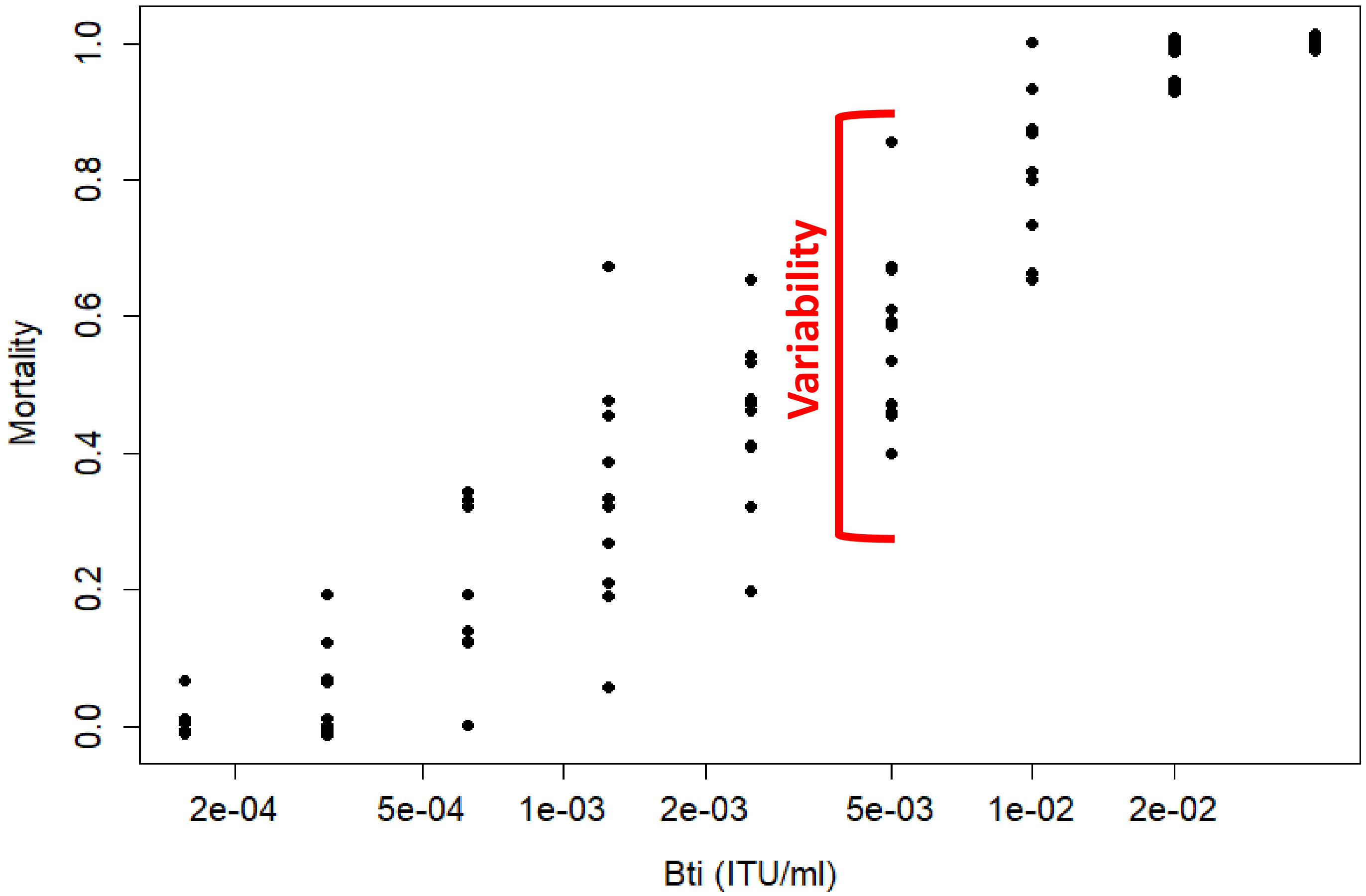


- LC-99 is the pesticide concentration where 99% of individuals from a susceptible colony would die
- Resistance ratios (comparing LC values) can be used, but require more specimens

Resistance can be defined by a threshold (diagnostic) concentration, dose, time, or resistance ratio based upon a susceptibility curve

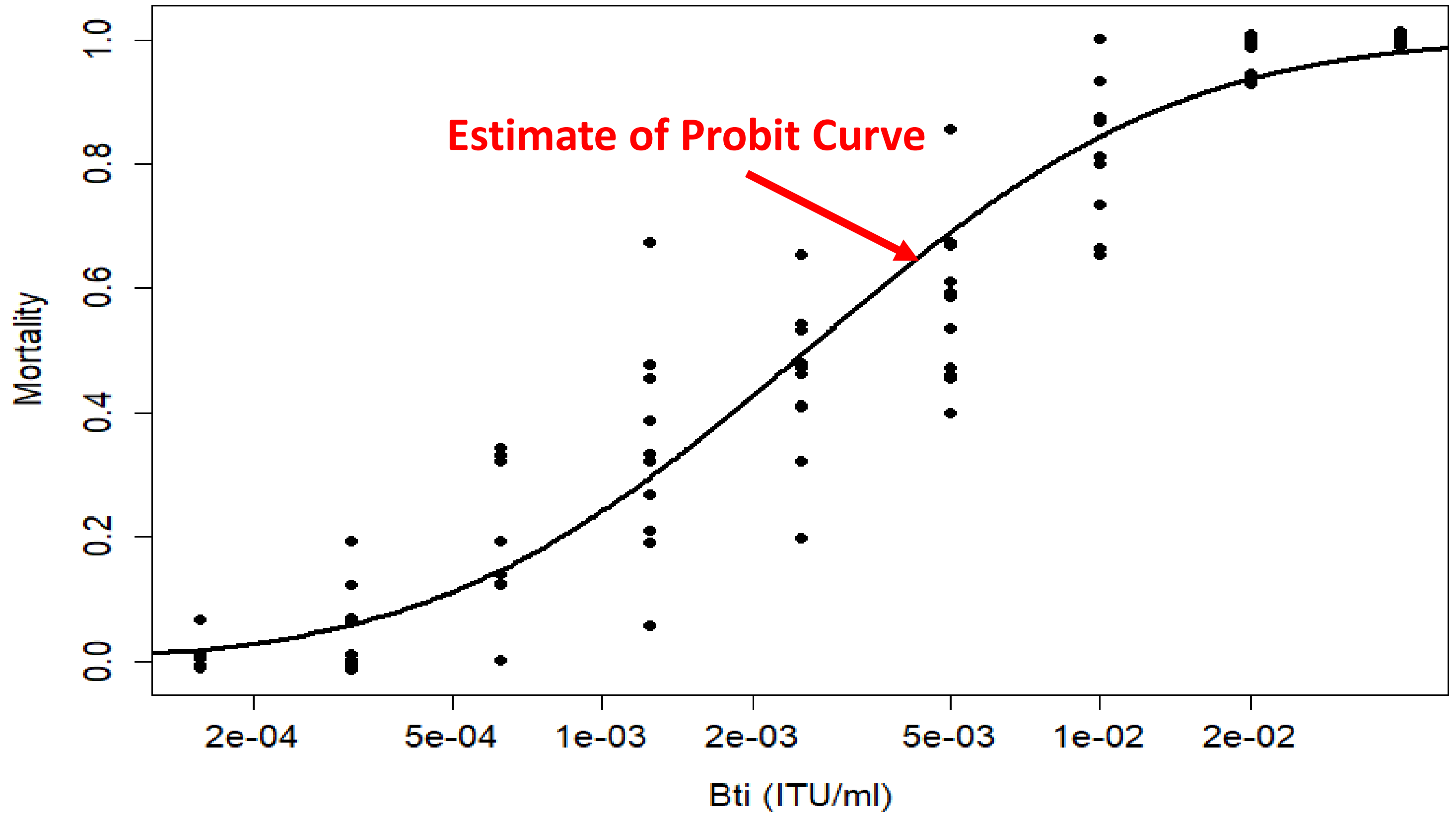
Susceptibility Curves

Bti Susceptibility Curve



Susceptibility Curves

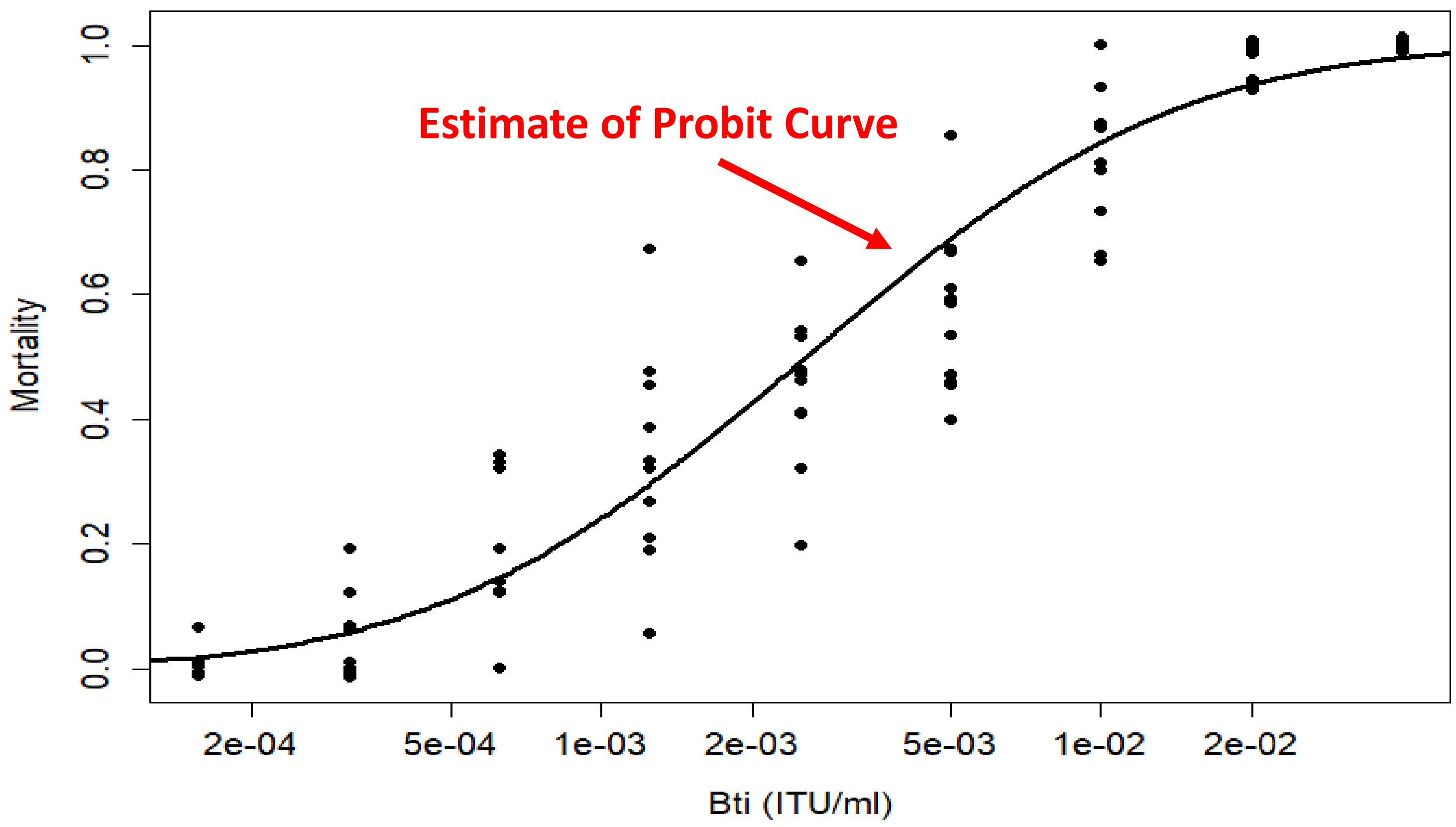
Bti Susceptibility Curve



Probit analysis: a type of regression used to analyze a dose-response curve. Can be used to determine the pesticide concentration at which a give percentage of mosquitoes will die

Susceptibility Curves

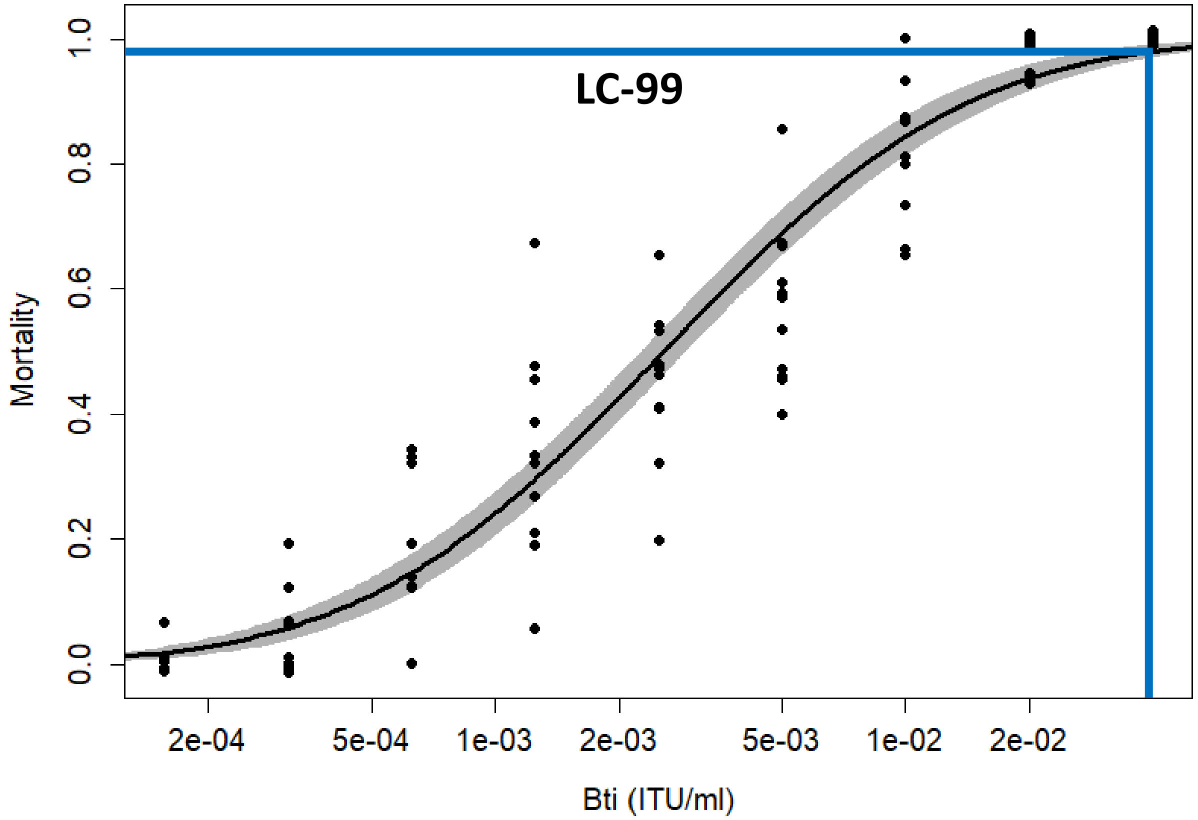
Bti Susceptibility Curve



Error **Replication**

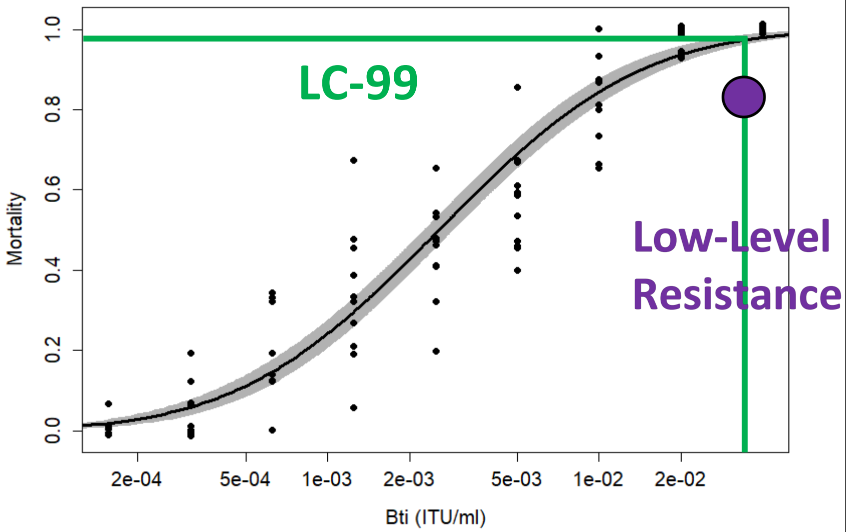
Susceptibility Curves

Bti Susceptibility Curve



Defining Resistance

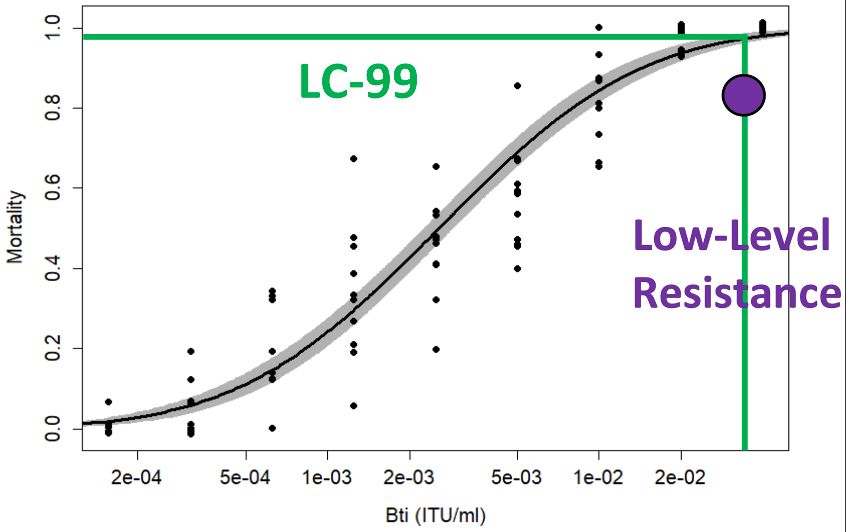
Larval Bioassays



	5x LC-99	10x LC-99
	Moderate Resistance	High-Level Resistance
	Mortality < 90%	Mortality < 90%

Defining Resistance

Larval Bioassays



5x LC-99

Moderate Resistance

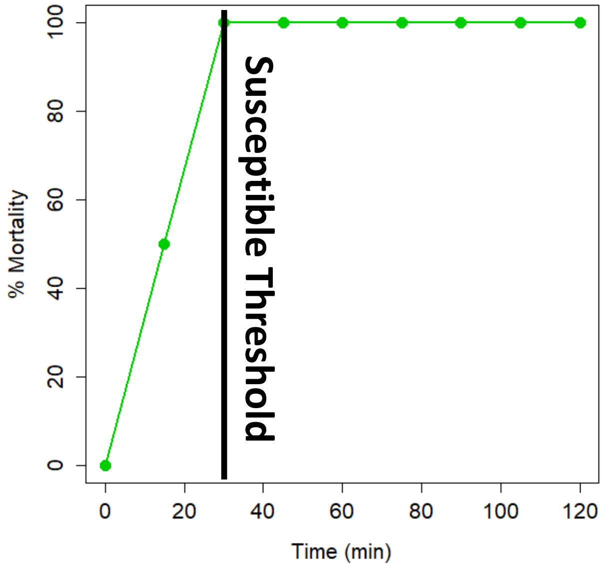
Mortality < 90%

10x LC-99

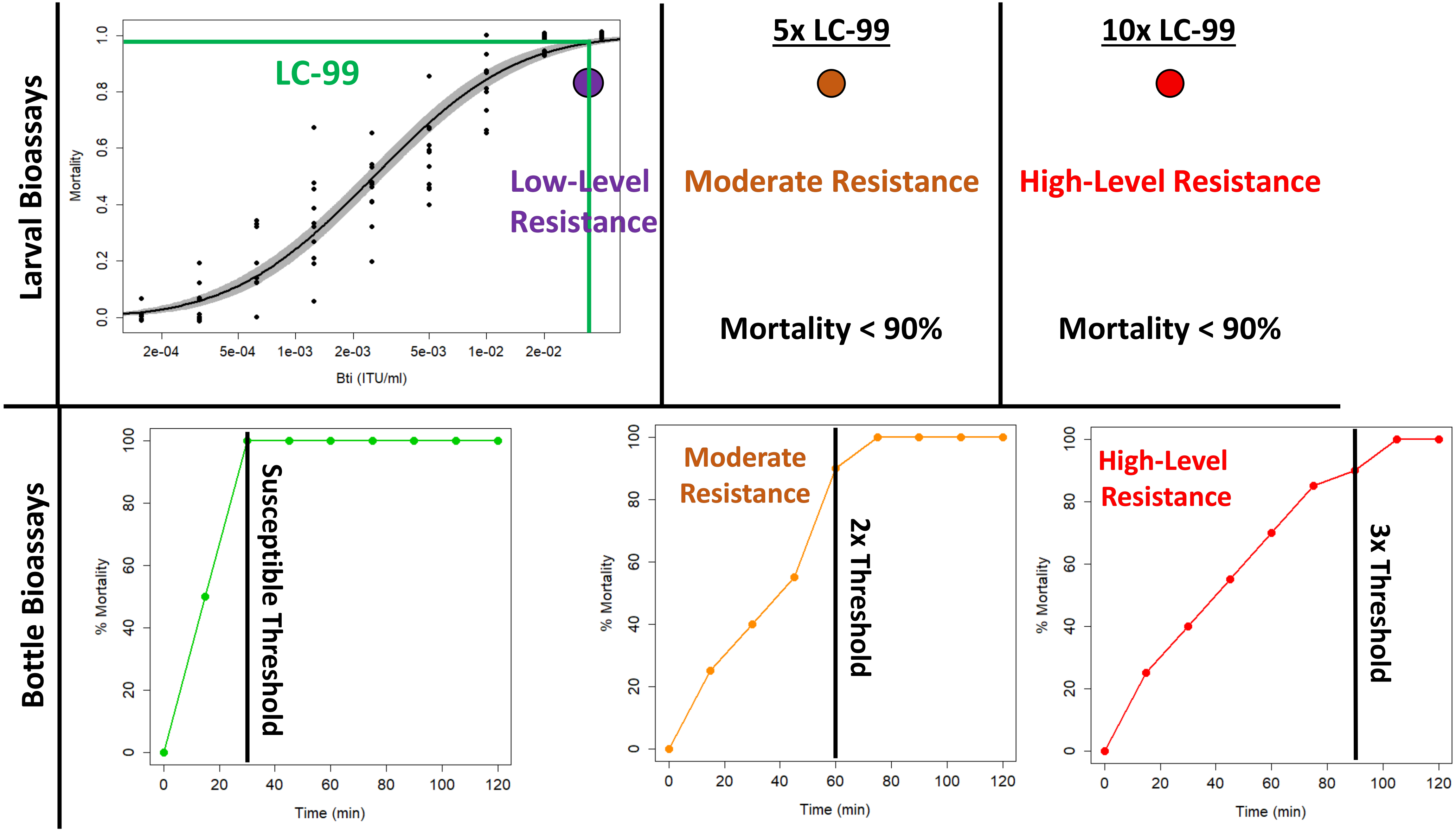
High-Level Resistance

Mortality < 90%

Bottle Bioassays

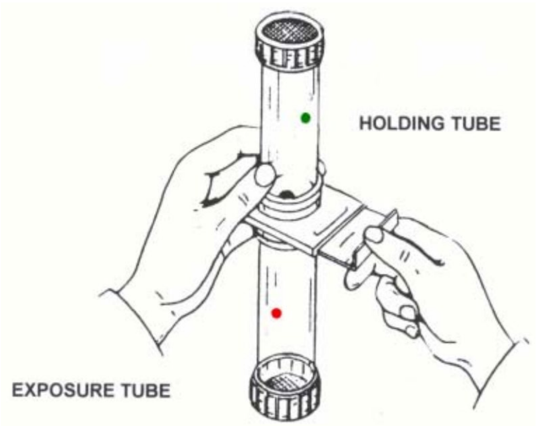


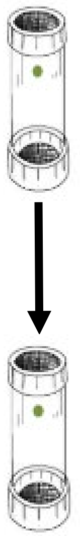
Defining Resistance



Common Detection Methods

WHO Test





Introduce mosquitoes to treated tube

After 1 hour transfer to clean tube

Hold mosquitoes in clean tube for 24 hours

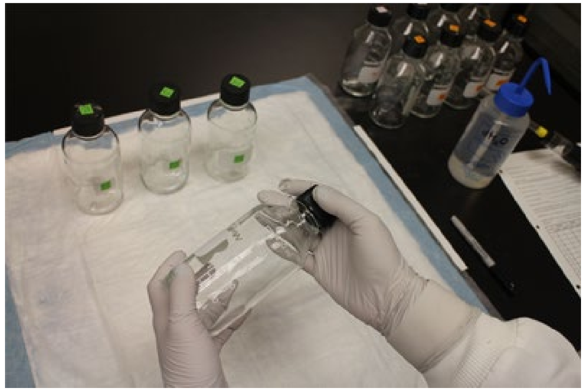
Insecticide class	Insecticide	Discriminating concentration (%) (1-hour exposure period)	5×[a] concentration (%) (1-hour exposure)	10×[a] concentration (%) (1-hour exposure)	Control paper
Carbamates	Bendiocarb	0.1	0.5	1	Olive oil
	Carbosulfan	0.4			Olive oil
	Propoxur	0.1			Olive oil
Organochlorines	DDT	4			Risella oil
	Dieldrin	0.4			Risella oil
		4			Risella oil
Organophosphates	Fenitrothion	1			Olive oil
	Malathion	5			Olive oil
	Pirimiphos-methyl	0.25	1.25	2.5	Olive oil
Pyrethroids	Alpha-cypermethrin	0.05	0.25	0.5	Silicone oil
	Cyfluthrin	0.15	0.75	1.5	Silicone oil
	Deltamethrin	0.05	0.25	0.5	Silicone oil
	Etofenprox	0.5	2.5	5	Silicone oil
	Lambda-cyhalothrin	0.05	0.25	0.5	Silicone oil
	Permethrin	0.75	3.75	7.5	Silicone oil
Phenylpyrazoles	Fipronil	2			Silicone oil
Synergist	Piperonyl butoxide	4			Silicone oil

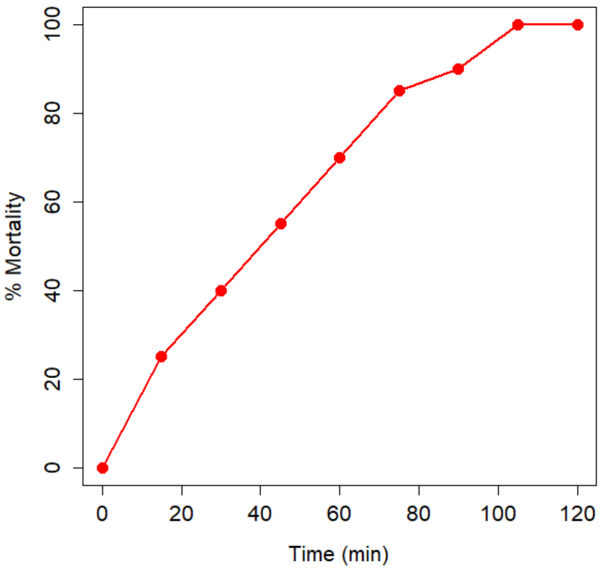
Test Procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2016

https://www.who.int/whopes/resources/who_cds_cpe_pvc_2001_2/en/

Common Detection Methods

CDC Bottle Bioassay

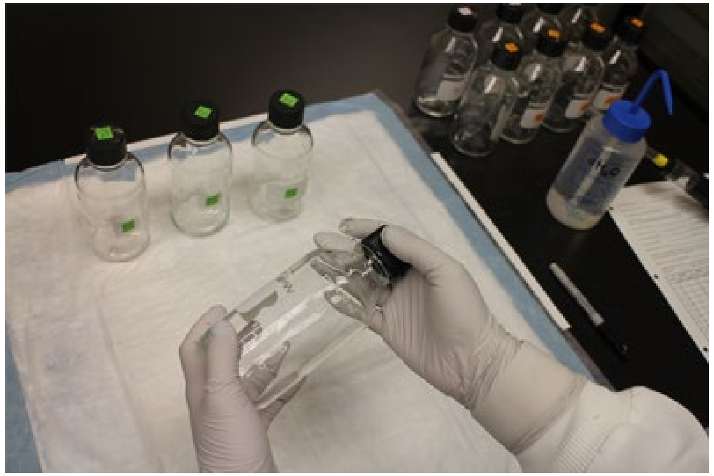




Chemical	Final Concentration/Bottle µg/bottle	*Ae. aegypti* REX colony	*Ae. albopictus* LC colony	*Cx. molestus* colony	*Cx. pipiens* NY/Chicago colony
		100% Mortality Expected (minutes)			
Chlorpyrifos	20	45	45	45	90
Deltamethrin	0.75	30	30	120+	45
Etofenprox	12.5	15	30	105	15
Fenthion	800	TBD	TBD	30	75
Malathion	400	15	30	30	45
Naled	2.25	30	30	30	45
Permethrin	43	10	10	30	30
Prallethrin	0.05	120+	120+	120+	60
Pyrethrum	15	15	30	120+	45
Resmethrin	30	5	10	30	15
Sumethrin	20	10	45	120	30

Comparing Methods

CDC Bottle Bioassay



- \+ Materials are cheap and easily accessible
- \+ Procedure is simple and easy to follow
- \+ Only one test to scale resistance level

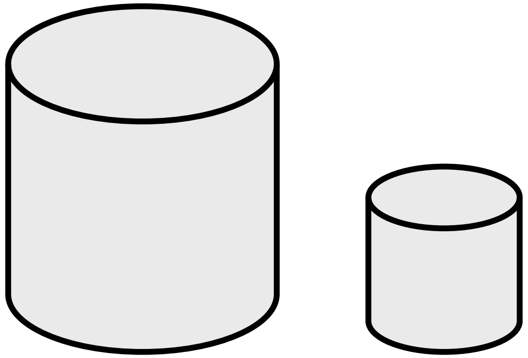
- \- Time consuming to collect data, if resistance is detected
- \- Level of resistance difficult to determine when using a diagnostic time

WHO Test

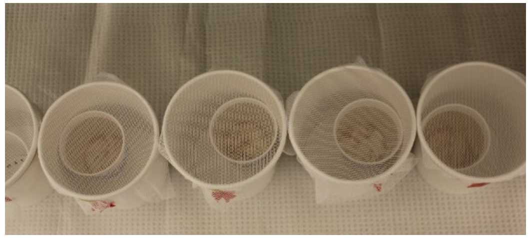
- \+ Only checked once (post-24 h) for each concentration used
- \+ Diagnostic doses are uniform and based on susceptibility curves

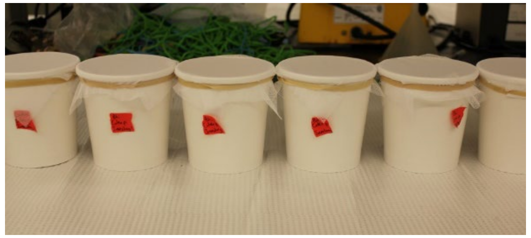
- \- Kits are difficult to obtain or build
- \- Must run multiple tests to detect different levels of resistance

Larvicide Bioassay

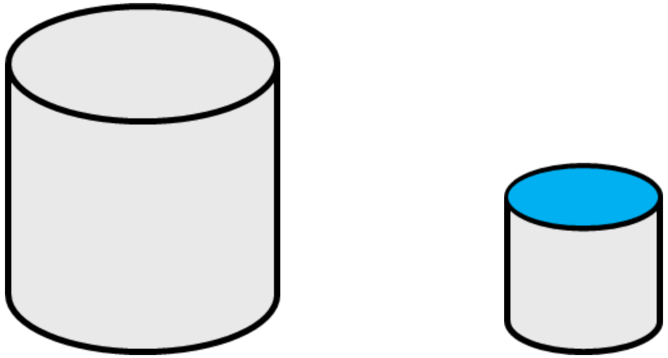


Use two wax-lined paper cups

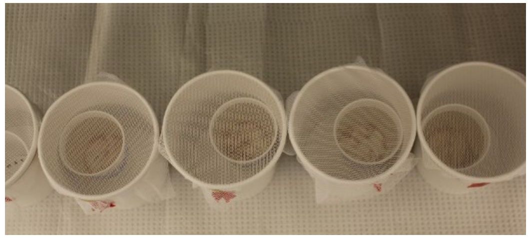


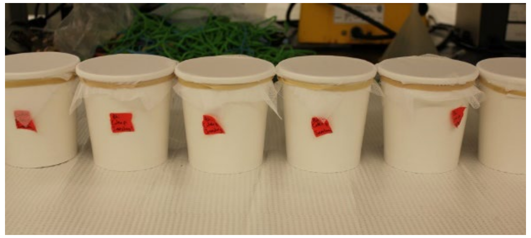


Larvicide Bioassay

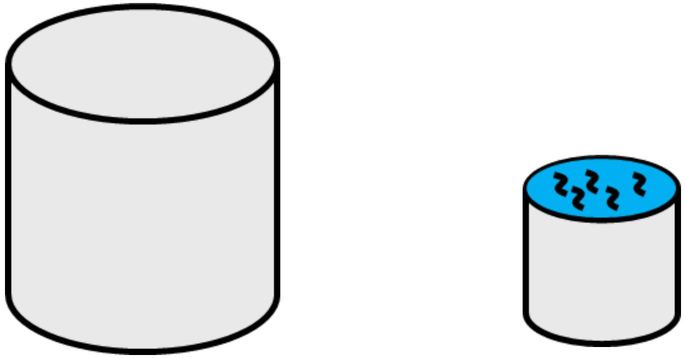


Fill the smaller cup with 74 ml of water and add 50 mg of fish food

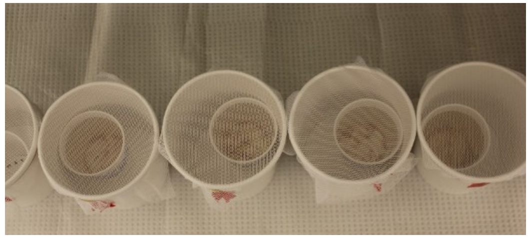


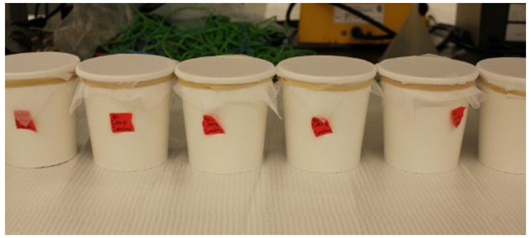


Larvicide Bioassay

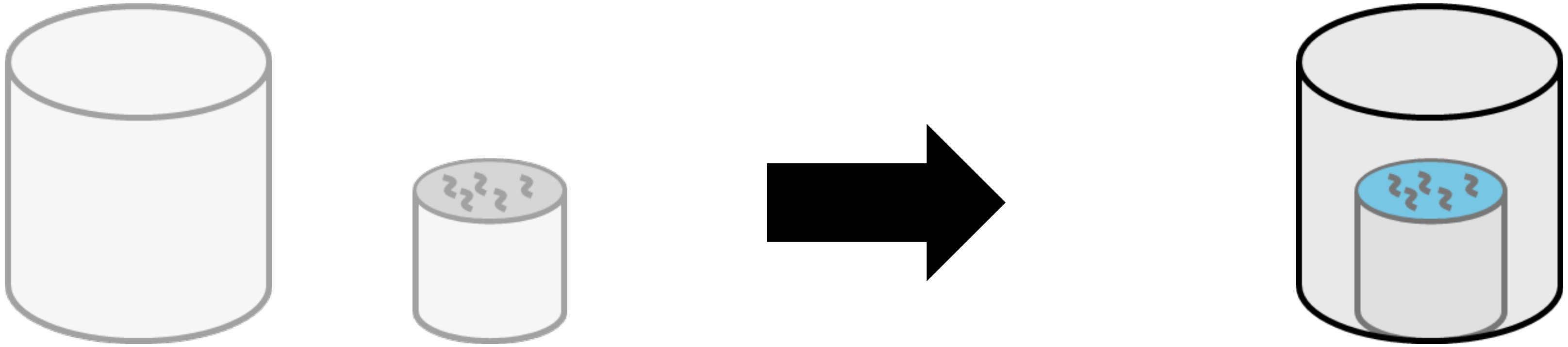


Add 15 4th instar larvae to the small cups

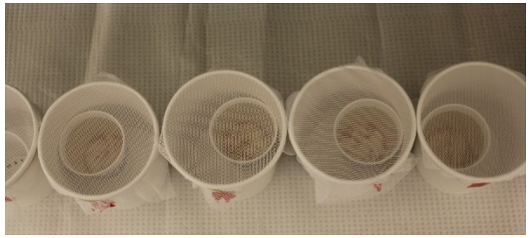


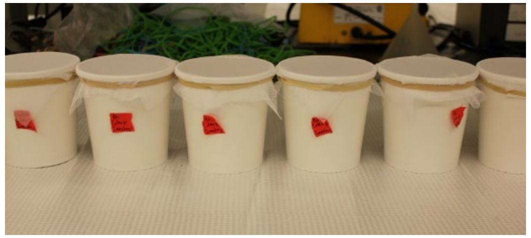


Larvicide Bioassay

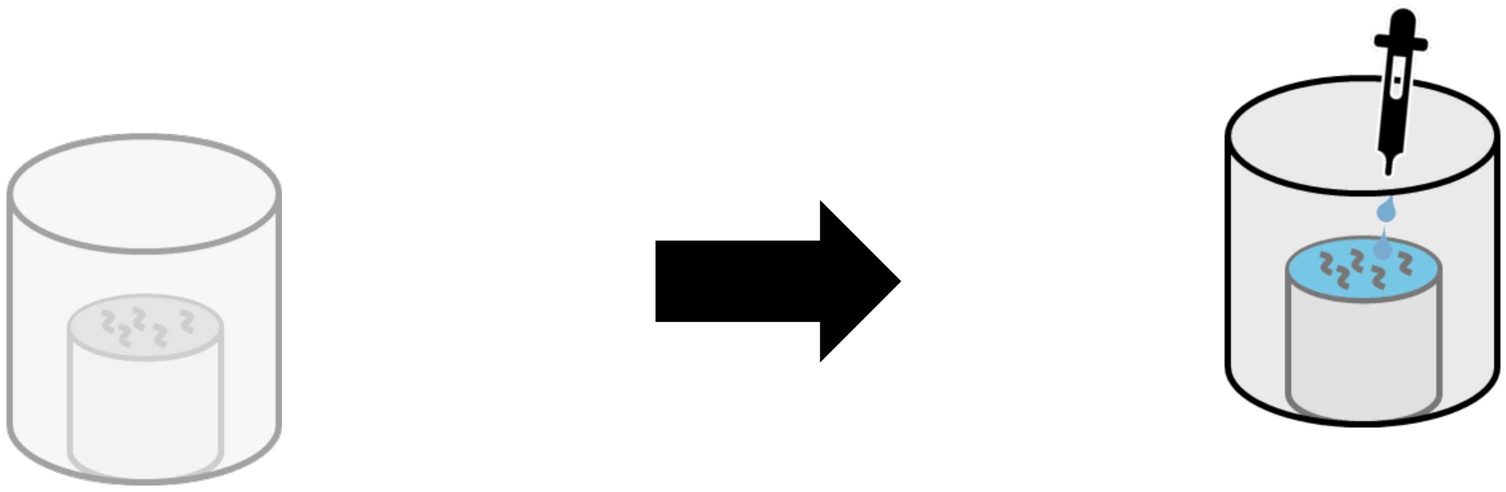


Place smaller cup
into the larger cup

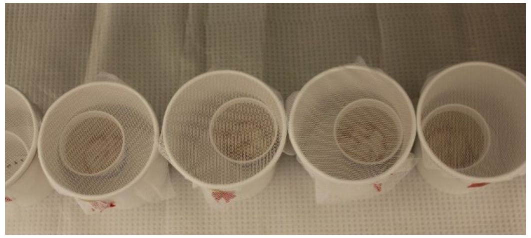


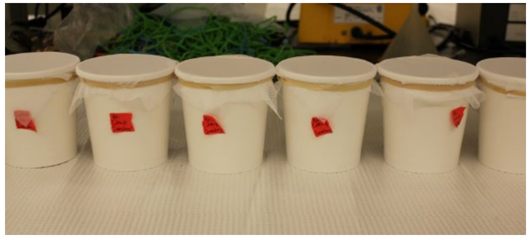


Larvicide Bioassay

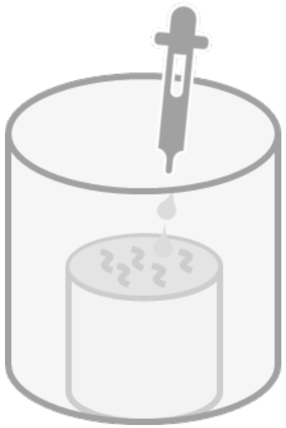


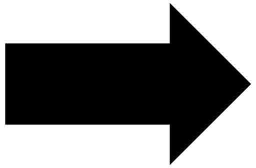
Add 1 ml of larvicide AI to the small cup containing water and larvae

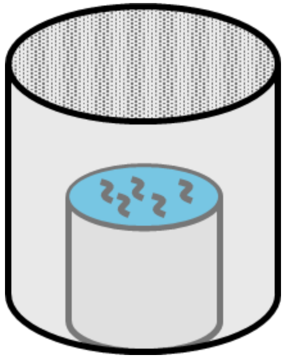




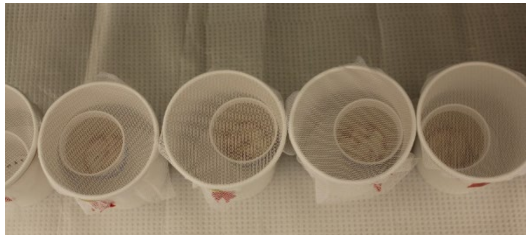
Larvicide Bioassay

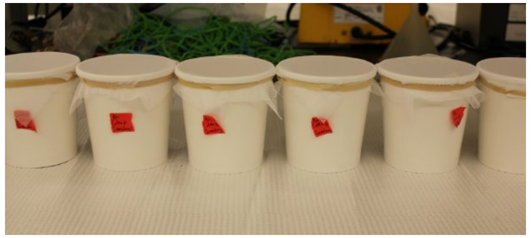




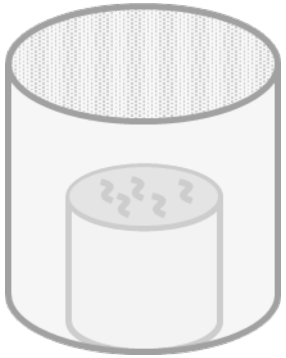


Place a piece of fine fabric
over the top of the larger cup





Larvicide Bioassay



Highest Concentration

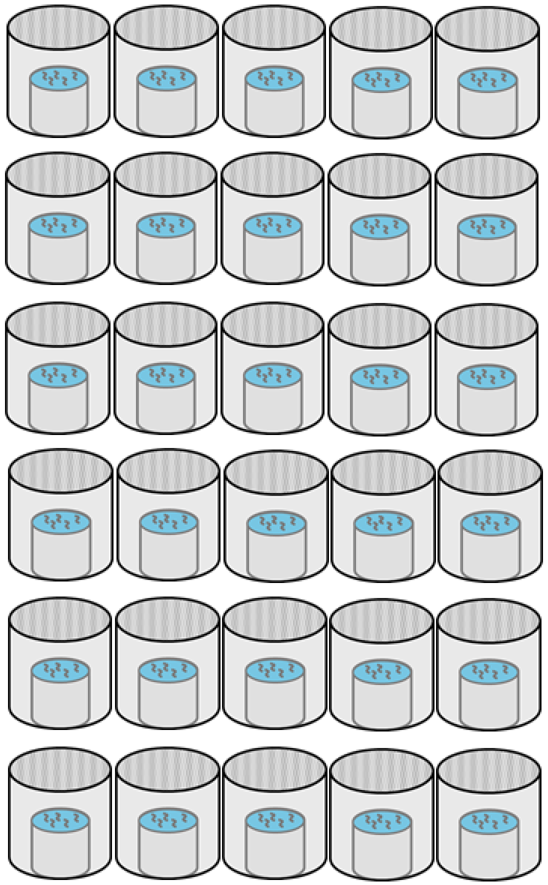
1:2

1:4

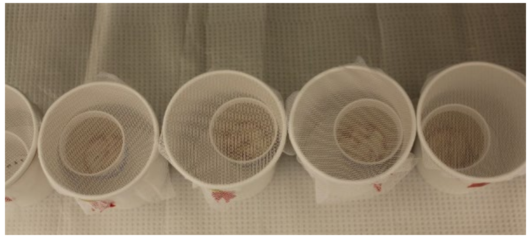
1:8

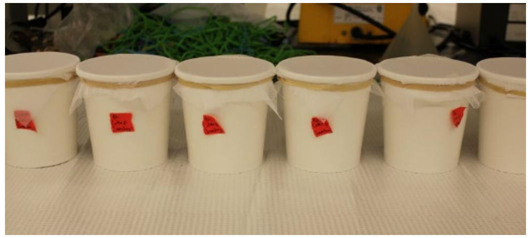
1:16

CTRL



Determine 5-6 concentrations to build susceptibility curve





Larvicide Bioassay

Highest Concentration

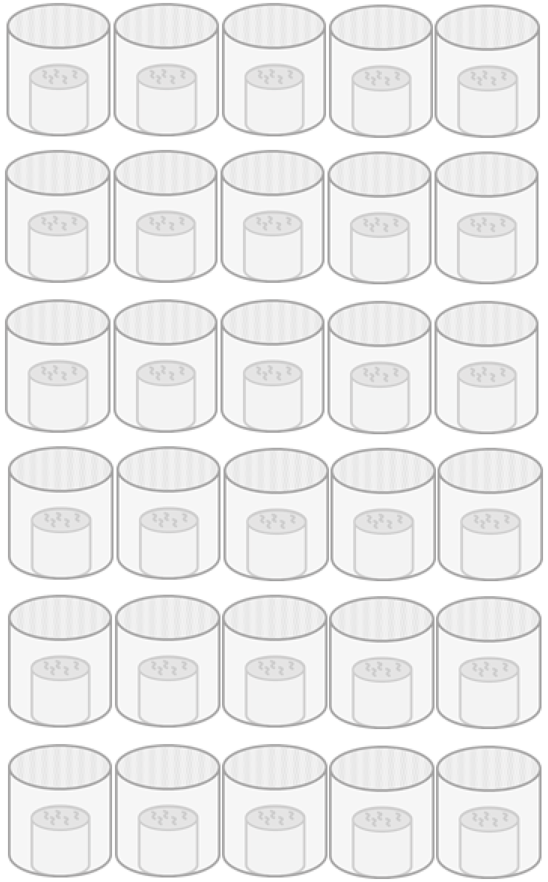
1:2

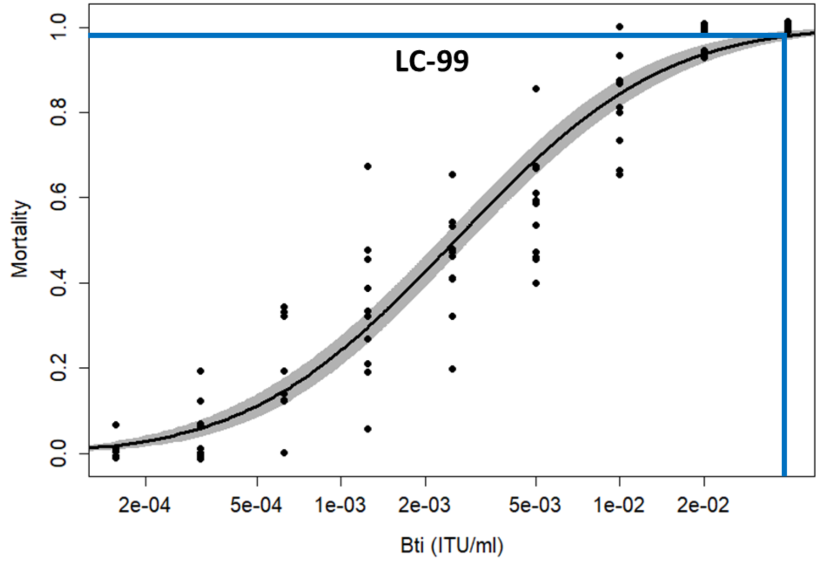
1:4

1:8

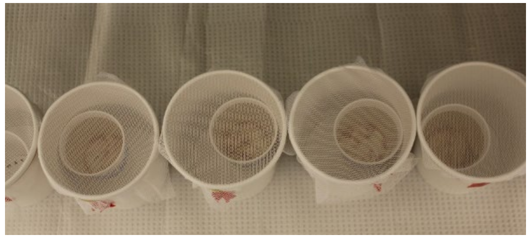
1:16

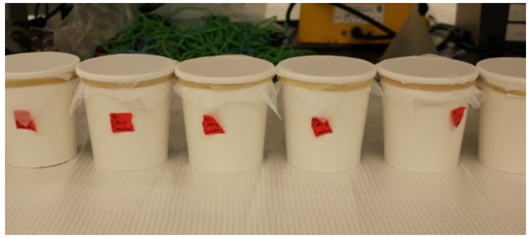
CTRL



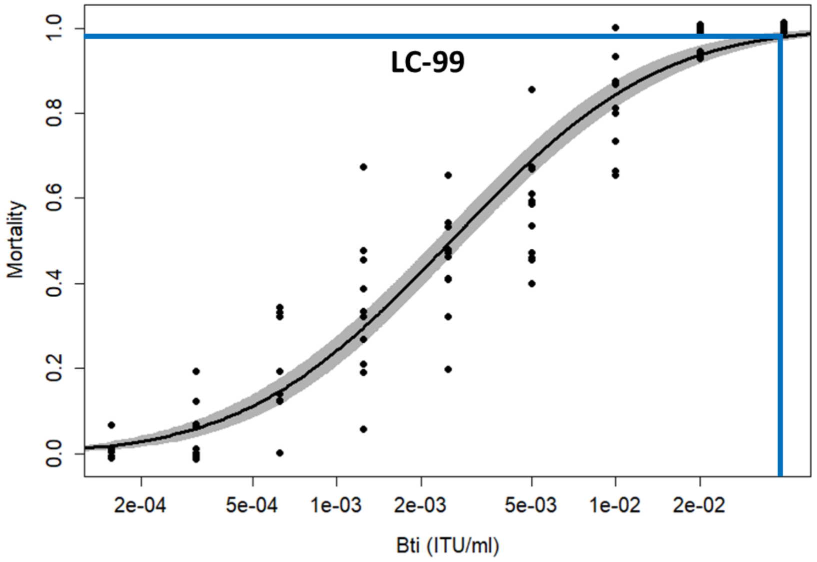


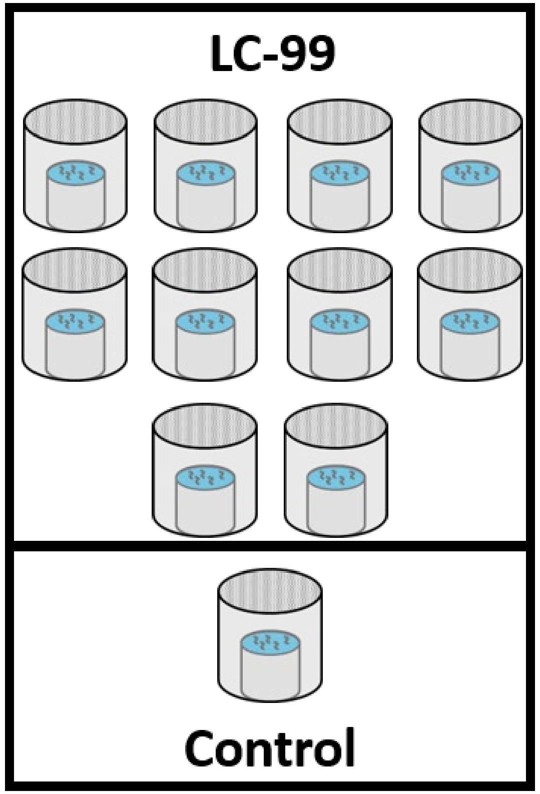
Conduct probit analysis to determine the diagnostic dose



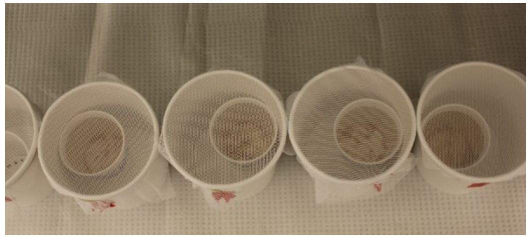


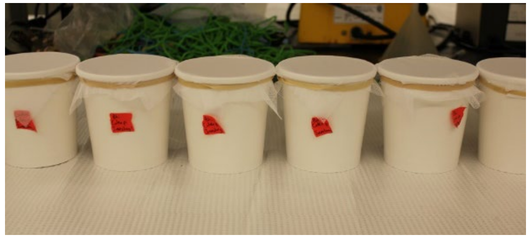
Larvicide Bioassay





Test field populations at the diagnostic concentration





Mosquito Collection for Assays

Egg Collection

BEST OPTION!

- + Age of mosquito known
- + Often easier to ID species
- - Requires some space for rearing

Larval Collection

- + Age of adults known
- - Age of larvae unknown
- - Each individual must be identified
- - Requires some space for rearing

Adult Collection

- + Only need to hold mosquitoes for short time
- - Age unknown
- - Cannot test larvae
- - Each individual must be identified

Factors Influencing Selection for Pesticide Resistance

Genetic

- Frequency and dominance of resistance genes
- Expression of genes
- Competition between individuals with and without resistance genes

Operational

Chemical

- Chemical nature of pesticide and similarity to other chemicals used historically
- Persistence o residues

Application

- Application threshold
- Life stage(s) targeted
- Mode of application
- Rotation of chemicals

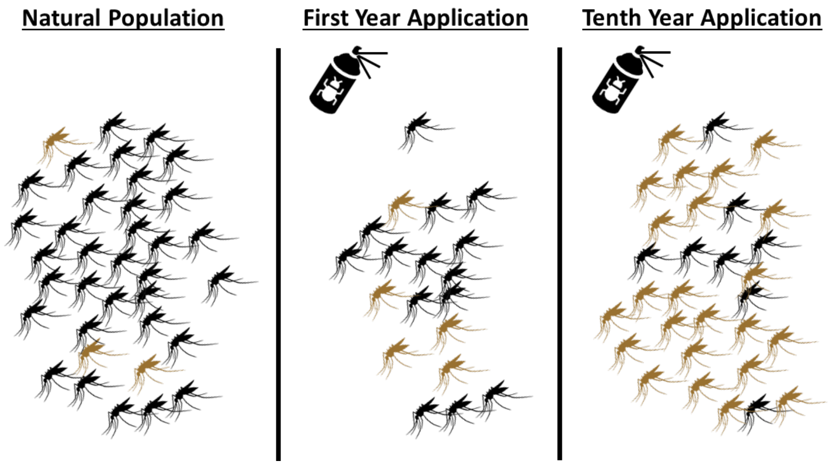
Biological

Reproductive

- Generation time and reproductive output
- Mate once, multiple times, or not at all

Behavioral

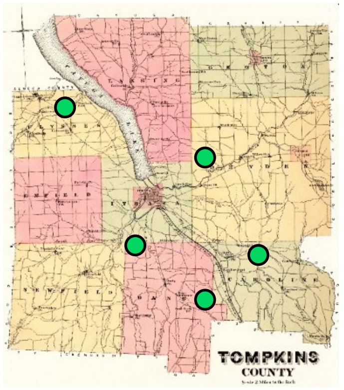
- Distance traveled
- Diet type (specialist / generalist)
- Shelter

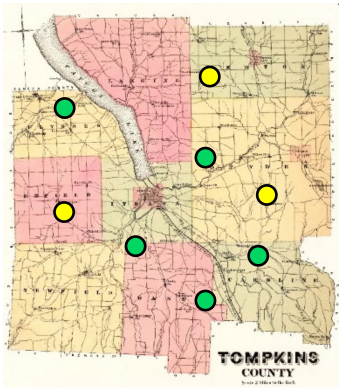


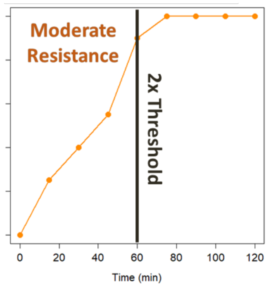
Managing Pesticide Resistance

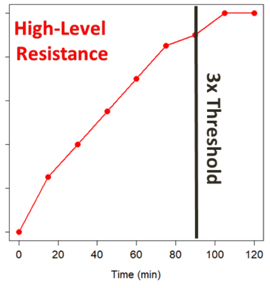
Diagnostic Dose / Time

- **Susceptible >97% mortality** → **Continue Testing Selected Sites**
  - Target subset of sites that experience high levels of control activity
- **Developing Resistance 96 - 90% mortality** → **Retest old sites and include new locations**
  - Retest populations to confirm resistance and try to test new sites
- **Resistant <90% mortality** → **Intensity Testing and Consider Alternate Control Methods**









Managing Pesticide Resistance

Management by Moderation

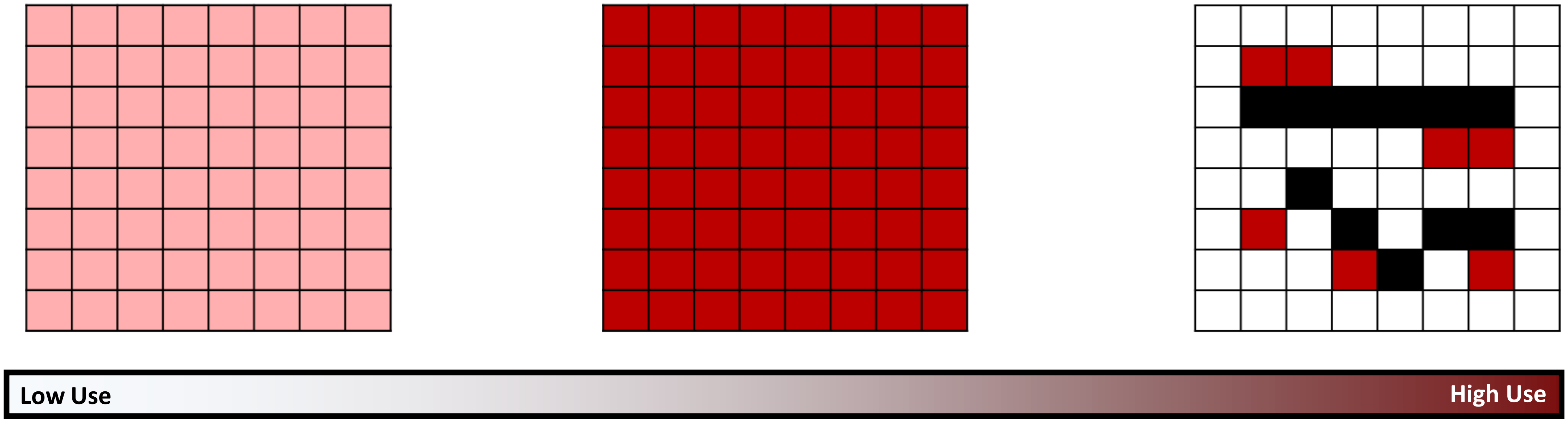
- Lower dosage and number of applications
- Use chemicals that are short lived
- Treat the minimal size area necessary

Management by Saturation

- Eliminating mosquitoes that have resistance genes at low levels before resistance emerges
- Use of synergists in the field

Management by Multiple Attack

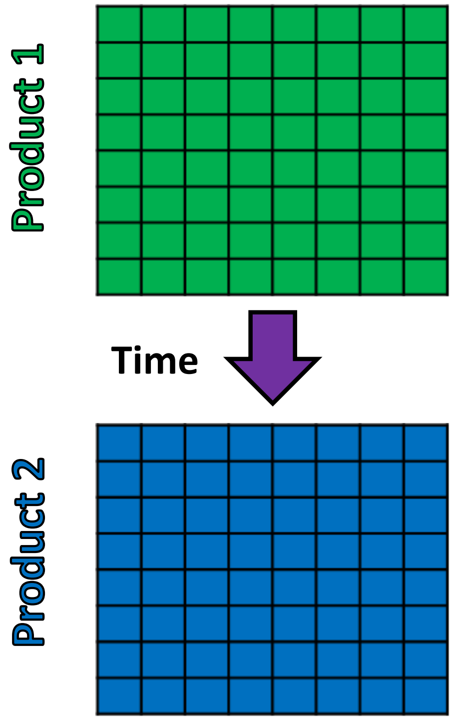
- Mixture of chemicals
- Alternation of chemicals: rotations, mosaics



Management by Multiple Attack

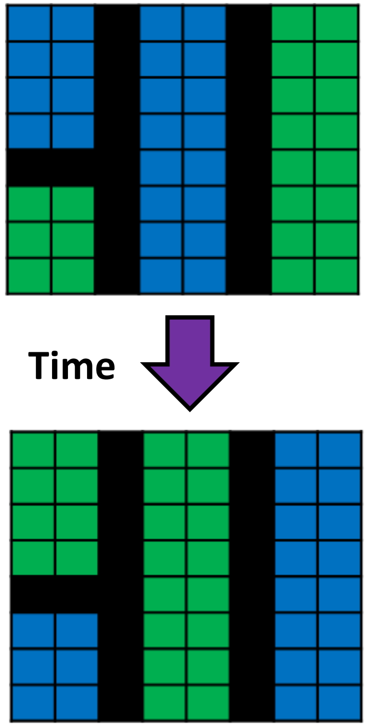
Rotation

- Switching between products with different modes of action
- This strategy should be paired with resistance monitoring for both products to detect emerging resistance



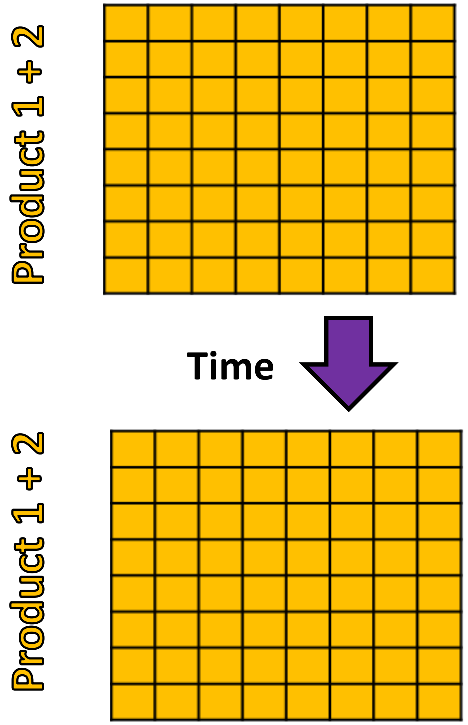
Mosaic

- Using different pesticides on geographically isolated mosquito populations
- Resistance testing for all populations and products is ideal for this method



Mixture of Insecticides

- Deploying a combination of two or more different pesticides to control mosquitoes at the same time
- Resistance testing for all products is necessary



Take it away John!

(We’ll have time for questions at the end of the webinar)

Establishing and Maintaining Mosquito Colonies



John Shepard

Northeast Regional Center of Excellence in Vector-Borne Diseases

Department of Environmental Sciences
Center for Vector Biology & Zoonotic Diseases
The Connecticut Agricultural Experiment Station
New Haven, CT

The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Establishing and Maintaining Mosquito Colonies

What do you need?

Space

Equipment

Effort

The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Resources

HHS Public Access

Author manuscript

Bio Protoc. Author manuscript; available in PMC 2017 October 24.

Published in final edited form as:

Bio Protoc. 2017 September 5; 7(17): . doi:10.21769/BioProtoc.2542.

Rearing of *Culex* spp. and *Aedes* spp. Mosquitoes

Elizabeth Kauffman[1], **Anne Payne**[1], **Mary A. Franke**[1], **Michael A. Schmid**[2], **Eva Harris**[3], and **Laura D. Kramer**[1,4,*]

[1]Wadsworth Center, New York State Department of Health, Albany, New York, USA

[2]Rega Institute for Medical Research, Virology and Chemotherapy, Department of Immunology and Microbiology, University of Leuven, Leuven, Belgium

[3]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, California, USA

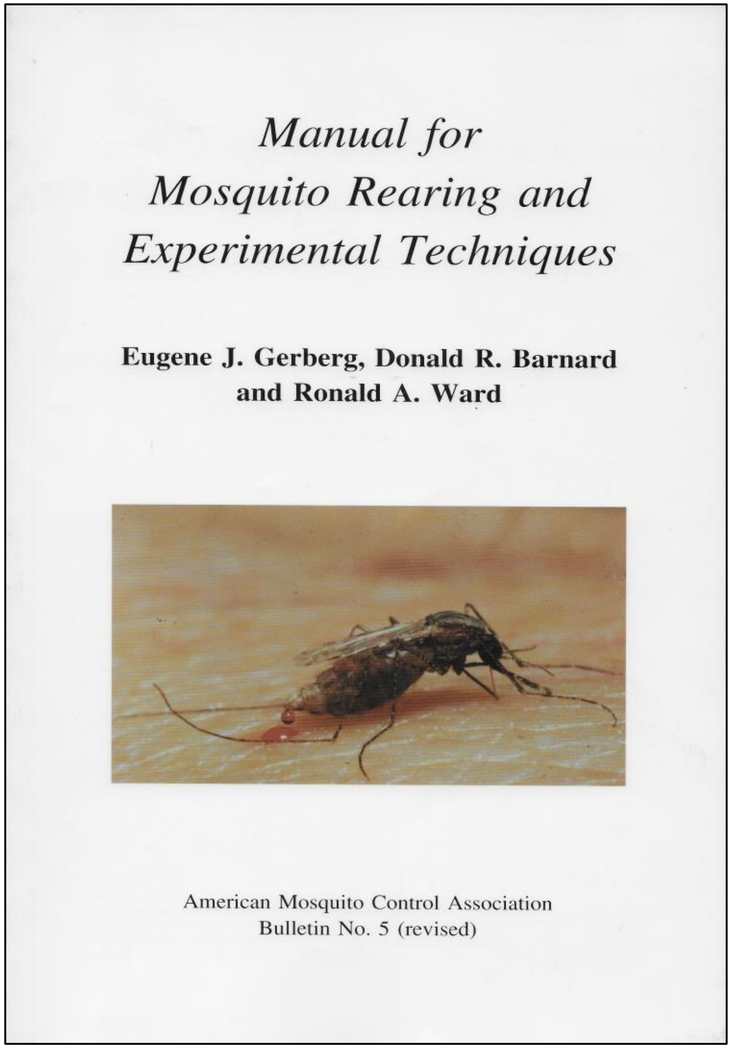
[4]School of Public Health, State University of New York at Albany, Albany, New York, USA

https://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC5654580&blobtype=pdf

Open Access

- **List of Supplies**
- **Techniques Covered in Detail**

Resources



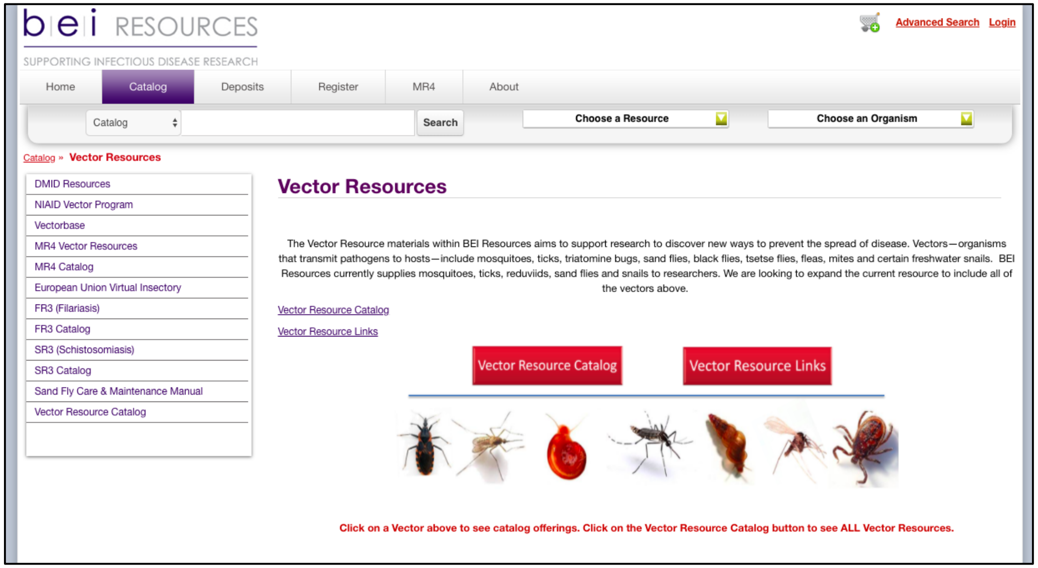
Everything you need to know

Compiles information and references from almost any colony work performed and documented up to early 1990s

Currently available from AMCA for purchase ($28)

https://www.mosquito.org/store/ViewProduct.aspx?id=9520947

BEI Resources



https://www.beiresources.org/

- **NIAID**
- **Repository**
- **Registration Required**
- **Eligibility**
- **Permits**
- **4 Species available**

Methods in *Aedes* Research (26 pp):
https://www.beiresources.org/Portals/2/VectorResources/Methods%20in%20Aedes%20Research%202016.pdf

Methods in *Anopheles* Research (408 pp):
https://www.beiresources.org/Portals/2/VectorResources/2016%20Methods%20in%20Anopheles%20Research%20full%20manual.pdf

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Facilities

Short Term

- Dedicated Bench space
- "In Season"
- No control needed for temperature or photoperiod
- Area for trays of larvae and cages of adults
- May need to increase humidity for Adult Cages
  - Damp paper towels
  - Plastic sheeting

Long Term

- Dedicated space
  - Incubator or Growth Chamber
  - Walk-in Incubator or Climate Controlled Room
- Controls needed for temperature and photoperiod

Facilities

Growth Chamber or Incubator

- Free standing
- 1 or 2 colonies
- Temperature Control (25.5° C)
- Light Control (16:8 hr, L:D)
  - Fluorescent

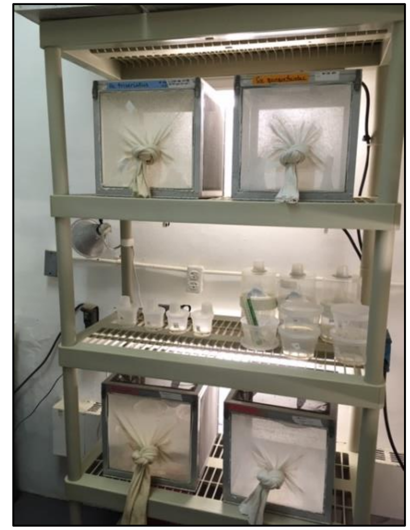
Insectary

- Dedicated space
- More Space
- Humidity Control

Insectary

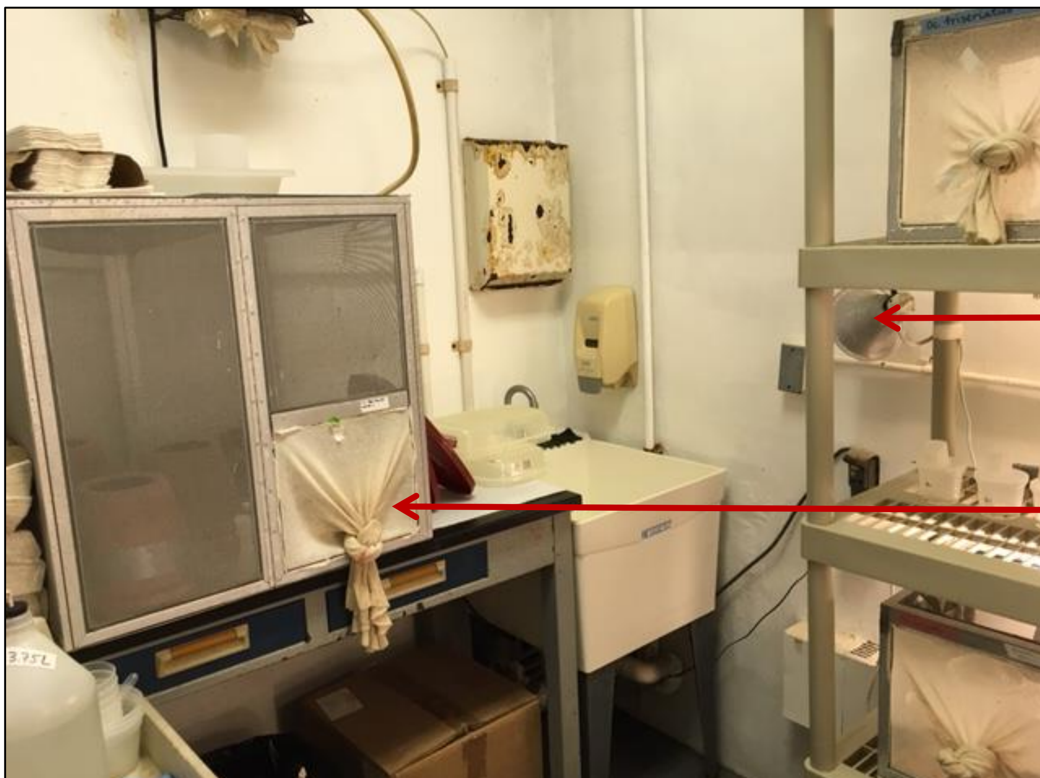
22 to 25° C
70% RH
16 hour light, 8 hours dark

- ***Overhead Lights***
  On 5:30 AM, Off 9:00 PM
- ***Lights on Shelf Units***
  On 6:00 AM, Off 9:30 PM



There are 2 Shelf Units in the room.

Each shelf has a light fixture



Dusk Simulation

Clip-on Work Light
10 W incandescent bulb
On 5:00 AM, Off 6:30 AM
On 9:00 PM, Off 11:00 PM

8 cu. ft. cage

Species

Multivoltine Species

- **Recommended**
  - *Culex pipiens** or *Cx. quinquefasciatus**
  - *Aedes albopictus**
  - *Aedes aegypti**
  - *Aedes triseriatus**
  - *Cx. pipiens* form *molestus* (autogenous)*
  - *Aedes atropalpus* (autogenous)#

Larvae Develop in Containers

- **More difficult**
  - *Culiseta melanura**
  - *Culex restuans*
  - *Culex salinarius* #
  - *Culex tarsalis* #
  - *Aedes japonicus*#
  - *Anopheles quadrimaculatus*

Univoltine Species

- **Can sometimes rear larvae to adult**
- **Extremely difficult to colonize**

* *= currently maintained at CAES* # *= previously maintained at CAES*

Specimen Collection

Ovi-Trap

- 32 oz. "Casino Cup"
- 38 lb. Seed germination paper
- *Aedes*/*Ochlerotatus* species
- ID larvae as 2^{nd}, 3^{rd} or 4^{th} instar

Photo: Greater Los Angeles County VCD

Container

- Gravid Trap Infusion as bait
- *Culex* egg rafts
- ID larvae as 1^{st} or 2^{nd} instar

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Specimen Collection

Dipper

- Multiple *Aedes/Ochlerotatus* species
- Vernal pool, salt marsh, floodwater
- ID larvae
- Rear to Adult in field collected water
- May need to feed up to 4th instar

Photo: John W Hock Co.

Mechanical Aspirator

- Backpack Aspirator
- Similar to Landing Bite Counts
- Mated Adult Females
- ID females (immobilized by chilling)
- Offer bloodmeal
- Collect eggs, hatch, rear larvae

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Rearing Larvae

- Larger pans work best
- White background
- Surface Area > Volume

Inexpensive Options

24 oz. Take Out Container

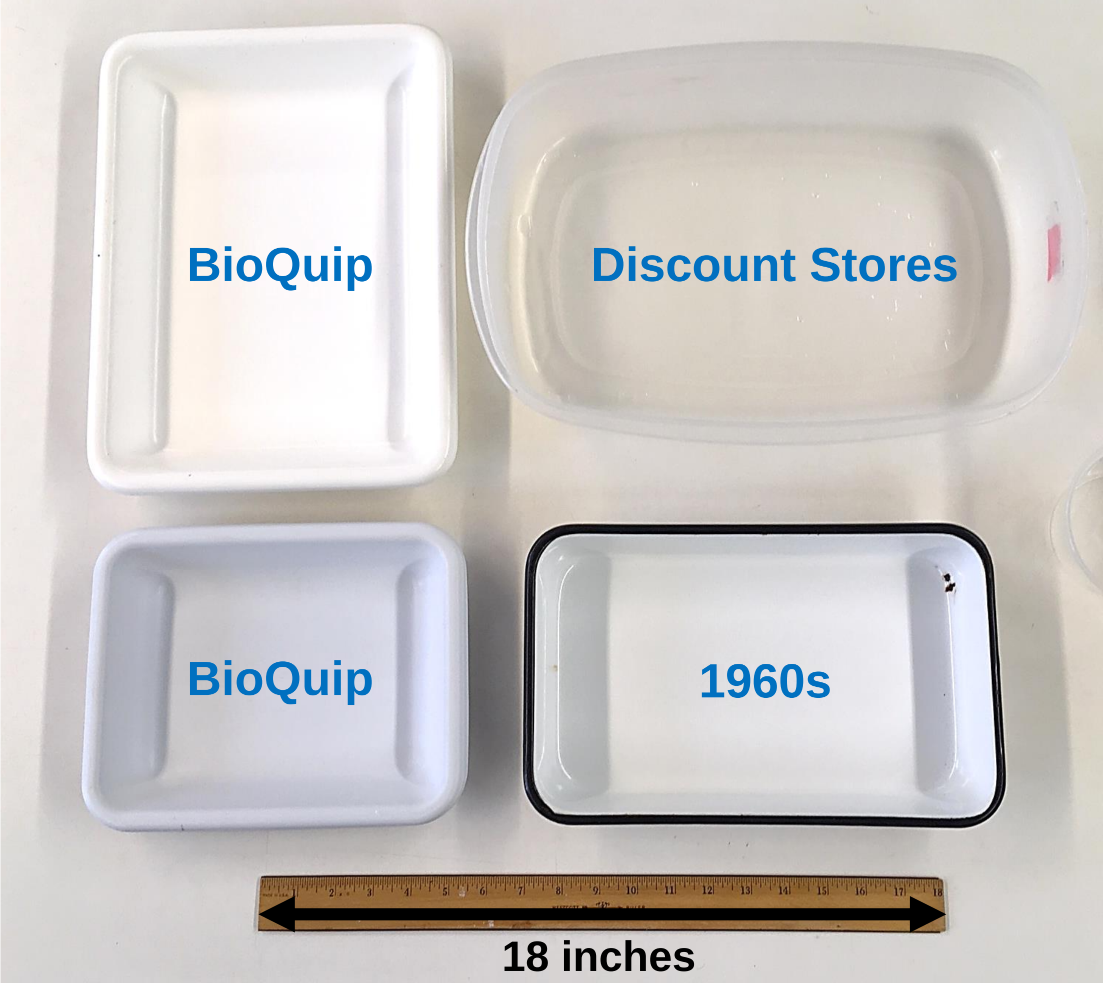
16 oz. Deli Container

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Rearing Larvae

- Larger pans work best
- White background
- Surface Area > Volume



Inexpensive Options

24 oz. Take Out Container

16 oz. Deli Container

Online : webstaurantstore.com

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Larval Diets

Multiple Options

- TetraMin Tropical Fish Flakes
  - Finely Ground
  - Inexpensive, readily available

- Liver Powder: Brewer's Yeast (3:2 by weight)

- Pet food (Finely Ground and Sifted)
  - Koi Food
  - Dog Food
  - Cat Food
  - Rabbit Food Pellets
  - Rat Chow

- Various Combinations of the Above

- Useful to mix in a suspension (slurry)
  - Make fresh for use or store 1-2 days

Hatching Eggs

Water

- Distilled
- Reverse Osmosis
- Conditioned Tap (let sit at least 24 hr)

Add water (about 4 cm deep), eggs, larval food

- General rule = 1 larva/1-2 cm^3 water (up to 5 larva/cm^2) OR LESS

***Aedes* Eggs: Flooded Friday, Larvae 2nd or 3rd instar on Monday**

The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Hatching Eggs

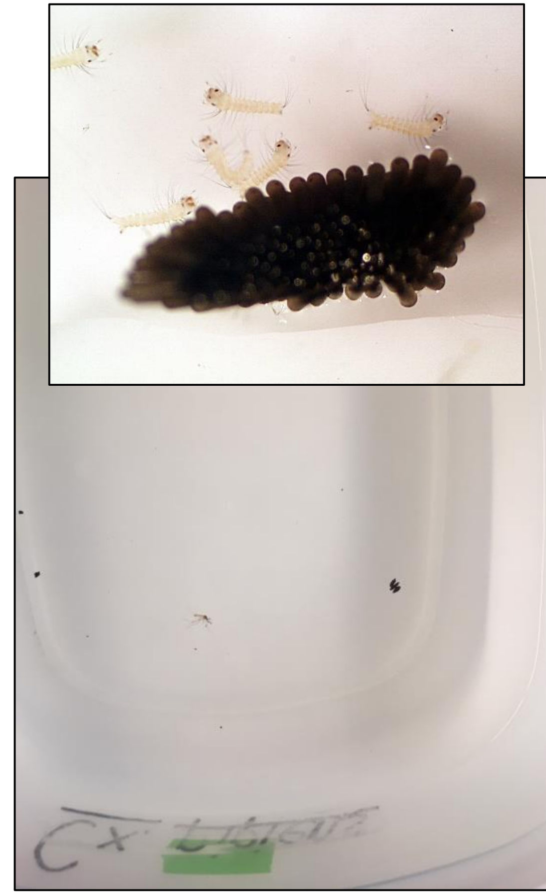
Culex egg Rafts

- Each raft = 50 to 200+ eggs
- Make sure to orient in “head down”

Add to water (2 – 3 cm deep), larval food

- Eggs hatch in 24 – 48 hrs





CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Feeding Larvae

Feed larvae Mon, Wed, Fri

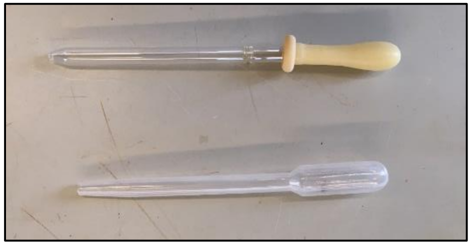
- Increase as Larvae develop from 1st to 3rd instar
- Skim surface with paper towel if "scum layer" develops
- Feed less if water is cloudy
- Decrease as when Pupae first develop

Transferring Pupae

Pick pupae as they develop

- 5 to 7 DAYS after hatching eggs

Pipettes



- Need to cut tip
  - Glass
  - Plastic

Transfer Pupae

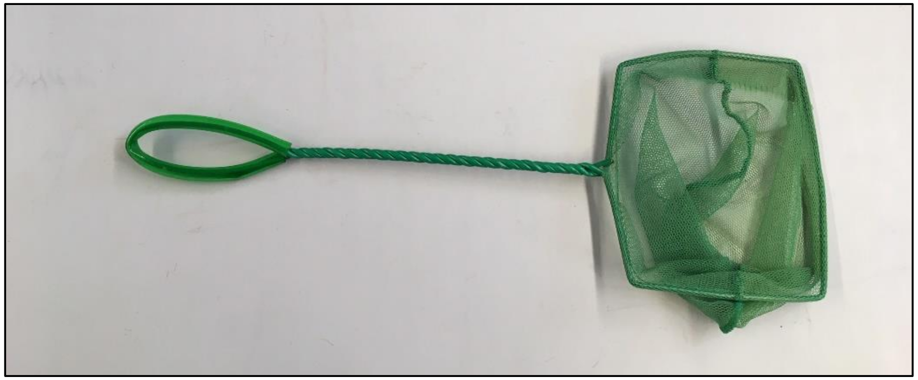
- Mosquito Breeder
- Deli Cup

Place into Cage

Optional Equipment

Fish Net

For Straining Pupae and 4th instars



The Connecticut Agricultural Experiment Station

Putting Science to Work for Society since 1875

Cages for Adults

BioQuip Products

- 30 x 30 x 30 cm (1 cubic foot)
- Metal
- Plastic - BugDorm

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Inside the Cage

Sugar Source

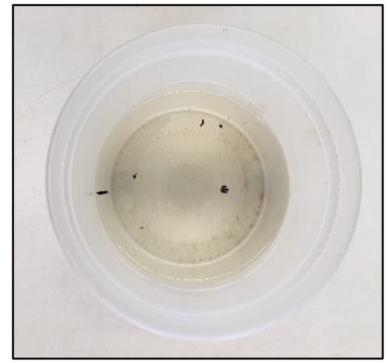
- Table Sugar
- 10% solution
- Cotton balls in 1 oz Cup
- Cotton Wick in 50ml flask

Raisins, Apple slices



Oviposition Dish

- Culex
  - Cup with water

- *Aedes*
  - Cup with water
    and fluted filter paper

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

BioQuip Products
Large Mosquito Breeder

The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Aspirators

For removing Adults from cage for Pesticide Resistance Testing

- **Battery Powered**

- **Lung Powered**

The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Blood Feeding

Required to Maintain a Colony Long-term

Live Animal

- **Institutional Animal Care and Use Committee (IACUC) Approval**
- **Detailed Protocols**
- **Need to maintain animals appropriately**
  - **Guinea Pigs**
  - **Button Quail**

Human Subjects

- **Regulated by US Department of Health and Human Services Common Rule (45 CFR 46, Subpart A)**
- **Institutional Review Board (IRB) Approval**
- **Occupational Activity**
- **Achee et al.** *Considerations for the Use of Human Participants in Vector Biology Research: A Tool for Investigators and Regulators.* Vector Borne Zoonotic Dis. 2015 Feb 1; 15(2): 89–102. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4340630/

Blood Feeding

Human Subjects

- Many IRBs in the United States will follow the OHRP and NIH definition of human subjects research
    - https://grants.nih.gov/policy/humansubjects/research.htm

- As a consequence, they will not consider direct mosquito feeding a human subjects activity that requires IRB approval, provided no individual data on participants is collected.

- It is recommended that investigators submit projects having a human feeding component to their appropriate IRB for determination that the proposed study activity should be deemed exempt.

Blood Feeding - Artificial

Blood from a supplier

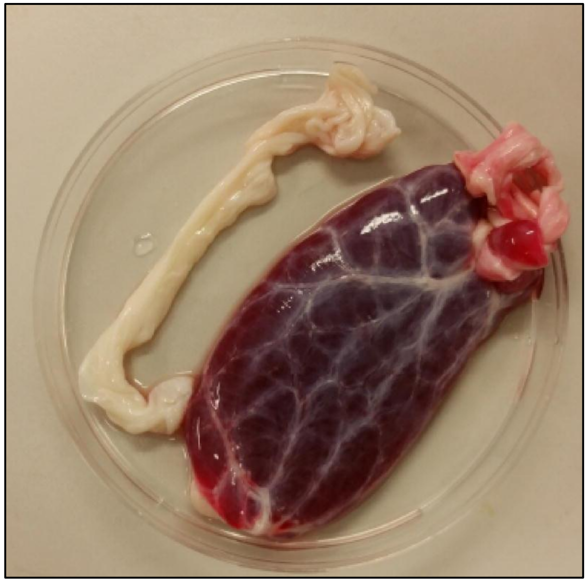
- Defibrinated
- Variety of blood available (sheep, cow, chicken, etc.)

Artificial Membrane

- Sausage Casing (sheep or pig intestine)

Blood warmed to 37°C (up to 40°C)

Sausage Casing Filled With Blood (r) and Unfilled
Kauffman et at. *Bio Protoc*

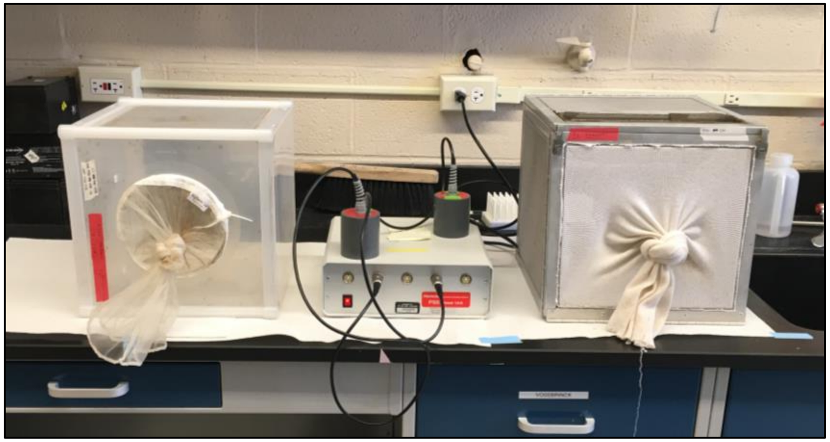


The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

Artificial Membrane Systems

Hemotek System

- **Parafilm or Casing as Membrane**



Glass Feeders

- **Parafilm or Casing as Membrane**
- **Requires Circulating Water (37-40°C)**

Discover Magazine: Will Betz/Seattle Biomed.

Vosshall Lab, Rockefeller Univ.

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

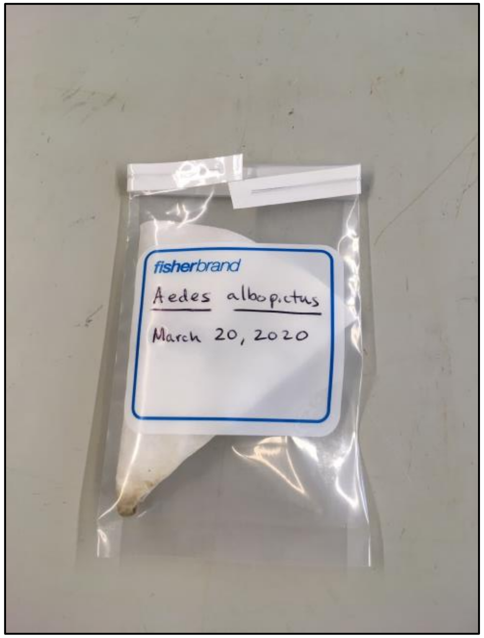
Storing Aedes Eggs

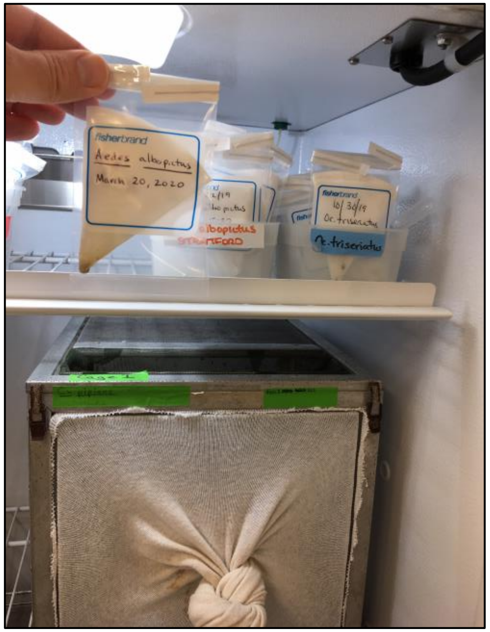
Oviposition 3-5 days after Blood Feeding

Dry on Paper Towel 15-30 mins

Fold with Eggs on the inside.

Place in Labeled Bag





Store for 1-6 months in Rearing Incubator or Insectary

CAES
The Connecticut Agricultural Experiment Station
Putting Science to Work for Society since 1875

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Questions?

John.Shepard@ct.gov

Questions?

Contact

nevbd@cornell.edu   jb766@cornell.edu

https://neregionalvectorcenter.com

John.shepard@ct.gov

https://portal.ct.gov/caes

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES