Notes From the Field: Elements of Mosquito Surveillance Related to Zika Virus

February 20, 2018
1:00-2:00 PM (ET)

Council of State and Territorial Epidemiologists

Webinar Housekeeping

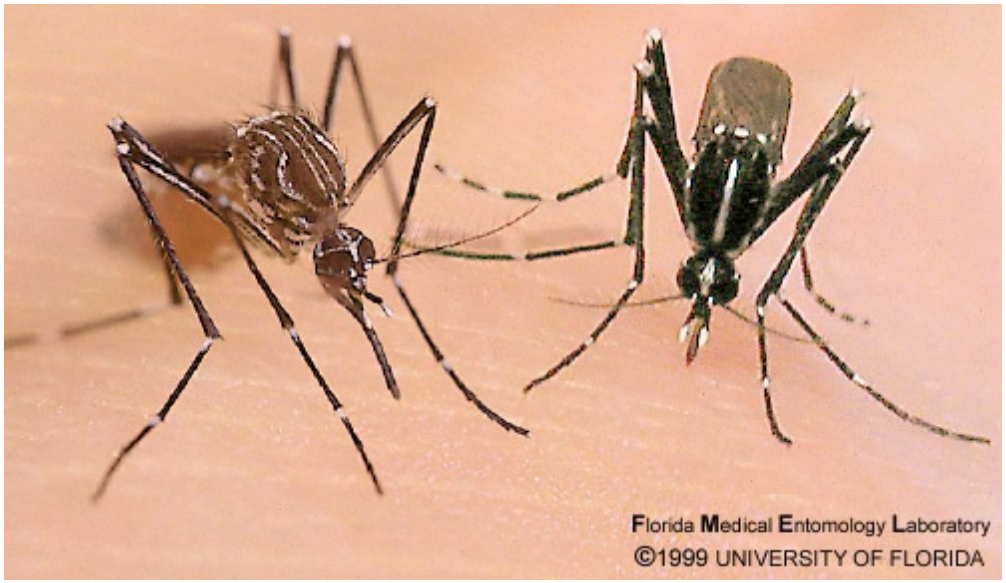
- **Today's webinar is being recorded**
  - The webinar recording and presentation slides will be available in the webinar library on CSTE's website: http://www.cste.org/?page=WebinarLibrary
- **All lines have been muted**
- **There will be a question-and-answer session at the end of the webinar**
  - To ask a question, please use the Q&A box on the right side of your screen

Webinar Objectives

Participants will:

- Registrants will be able to describe various strategies for conducting surveillance of Zika vector populations
- Registrants will be able to explain the importance of pesticide resistance testing for prevention and control strategies
- Registrants will be able to explain how public health jurisdictions can use the data from viral testing to create and support Zika virus prevention and control strategies

Mosquito Surveillance for Zika: Strategies, Necessary Resources and Prospects of Spread in the US



John Paul Mutebi, PhD

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U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Mosquito-Based Arbovirus Surveillance

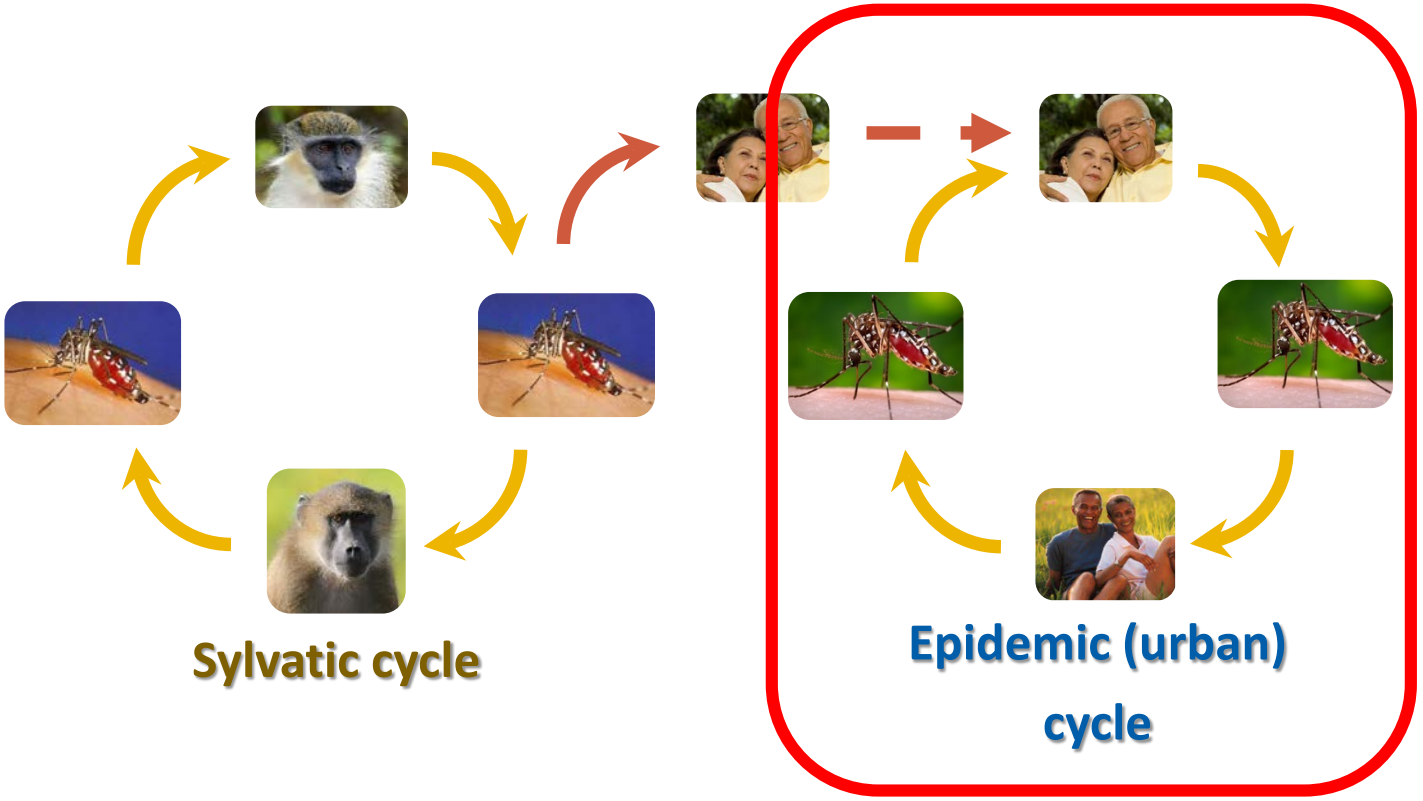
- consists of systematic collection of mosquito samples and screening them for arboviruses
- is the primary tool for quantifying the transmission of arboviruses and human risk
- aim = to predict changes in arbovirus transmission dynamics
- is an integral component of an integrated vector management (IVM) program

Principal Activities of a Mosquito-Based Arbovirus Surveillance Program

- Identifying and mapping larval habitats:
  - larval habitat information (essential for source reduction and larviciding)
  - estimates of future adult abundance
- Monitoring adult activity
  - species composition
  - species relative abundance (density)
  - age structure
  - infection rates

*Provides timely data for risk assessment

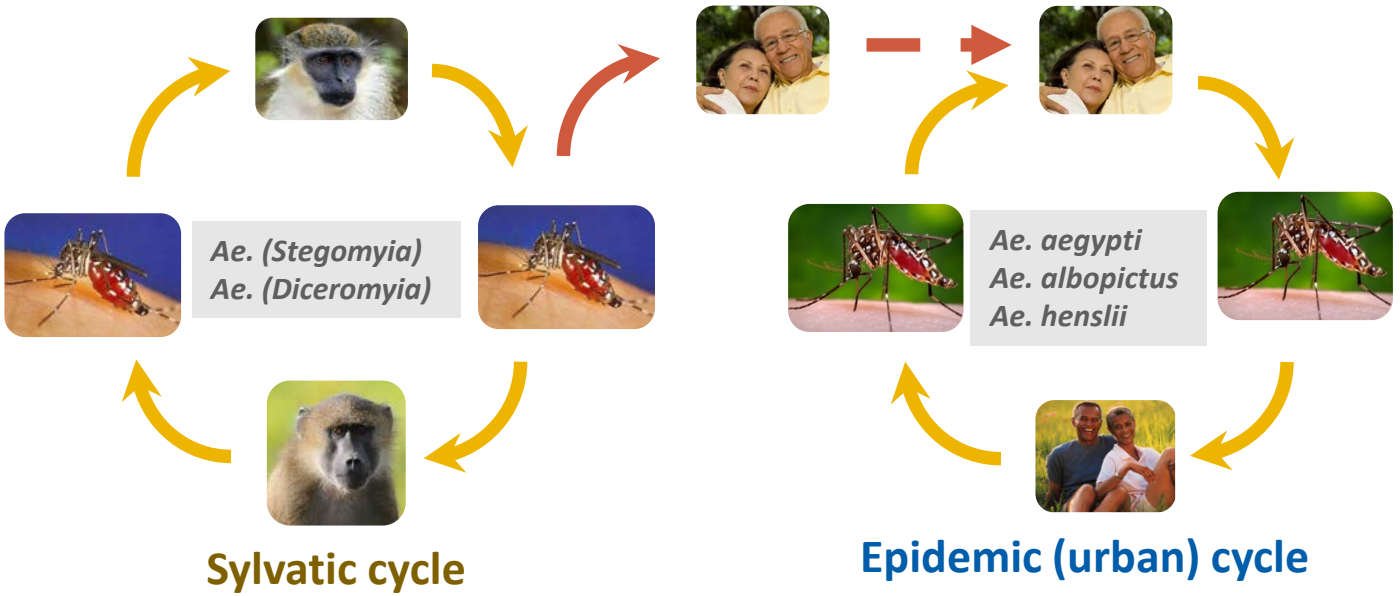
Zika Virus Transmission Cycles



ZIKV Vectors

1. *Ae. africanus*	Dick et al. 1952., Haddow et al. 1964, Wienbren and Williams 1958
2. *Ae. luteocephalus*	Lee 1969, Fagbami 1979
3. *Ae. vittatus*	Akoua-Koffi 2001, Diallo et al. 2011
4. *Ae. furcifer*	Akoua-Koffi 2001, Diallo et al. 2011
5. *Ae. taylori*	Diallo et al. 2011
6. *Ae. hensilli*	Ledermann et al 201)
7. *Ae. aegypti*	Olson et al. 1981, Akoua-Koffi 2001, Marchette 1969, Li et al. 2011
8. *Ae. albopictus*	Grard et al. 2012

Zika Virus Transmission Cycles



ZIKV Vectors

Diallo *et al*. 2014

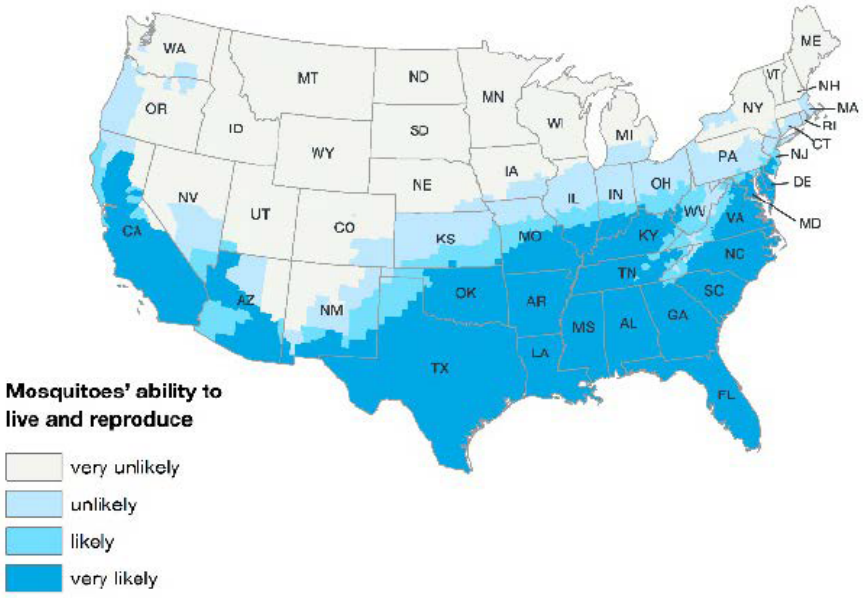
Species	Total collected	Proportion of the collection (%)	Females collected	Proportion of the collection (%)	Positive female pools	Minimum infection rate (‰)
Aedes aegypti	250	2.22	245	2.20	1	4.08
Aedes africanus	505	4.49	505	4.54	5	9.90*
Aedes dalzieli	1718	15.27	1718	15.44	2	1.16
Aedes furcifer	2966	26.37	2939	26.42	5**	1.36
Aedes hirsutus	34	0.30	34	0.30	2	58.82*
Aedes luteocephalus	1259	11.19	1259	11.32	5	3.97
Aedes metallicus	81	0.72	81	0.73	2	24.69*
Aedes taylori	422	3.75	395	3.55	2	5.06
Aedes unilineatus	38	0.34	38	0.34	1	26.31*
Aedes vittatus	1790	15.91	1728	15.53	3	1.74
Anopheles coustani	710	6.31	710	6.38	1	1.41
Culex perfuscus	22	0.19	22	0.20	1	45.45*
Mansonia uniformis	283	2.52	281	2.52	1	3.56
Others	1169	10.39	1169	10.51	0	
Total	11247		11124		30	

ZIKV Vectors in the CONUS

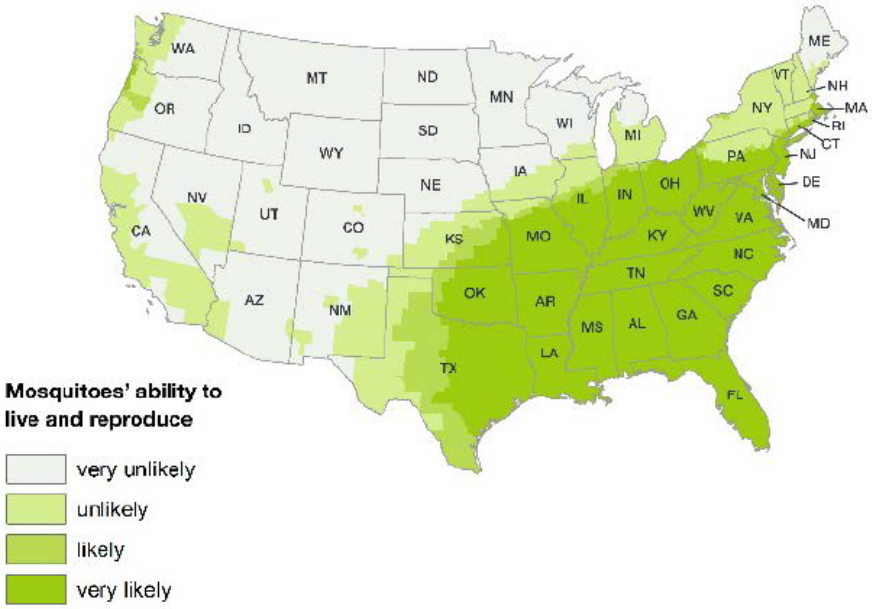
1. *Ae. aegypti* — Olson *et al*. 1981; Akoua-Koffi 2001; Marchette 1969; Li *et al*. 2012
2. *Ae. albopictus* — Grard *et al.* 2012

Estimated range of *Aedes aegypti* and *Aedes albopictus* in the United States, 2017*

Estimated range of *Ae. aegypti* in the United States, 2017*



Estimated range of *Ae. albopictus* in the United States, 2017*



Zika Virus Vector Surveillance Tools

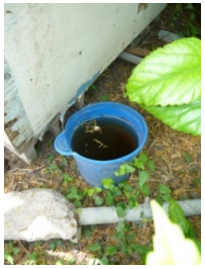
- Ovitraps (presence/absence; eggs/trap)

Zika Virus Vector Surveillance Tools

-Larval Surveillance

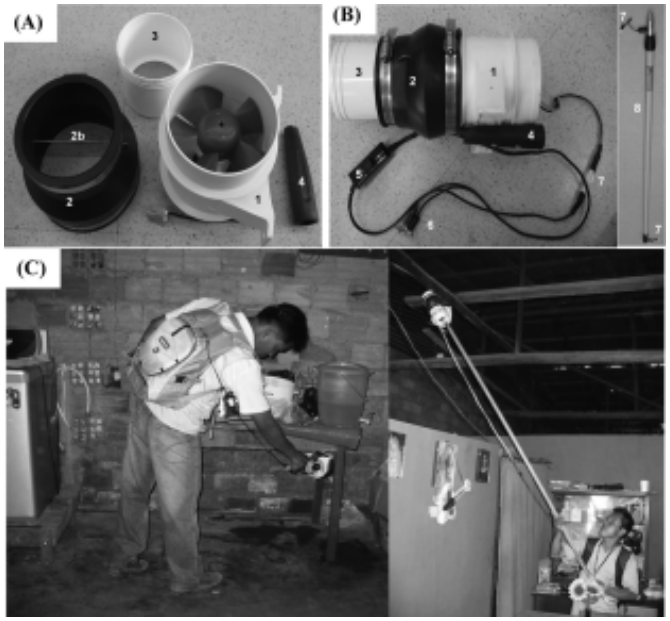
Container Aquatic Habitats For ZIKV Vectors

- Water-storage containers (barrels, jars, tanks, cisterns)
- Utensils (pails, tarps)
- Discarded containers (trash)
- Recreation objects (plastic pools, toys, boats)
- Ornamental (fountains, plant pots)
- Animal drinking pans
- Septic tanks
- Water meters
- Treeholes



Zika Virus Vector Surveillance Tools

- Electromechanical aspirators (Resting population)



CDC Autocidal Gravid Ovitrap (AGO)

(A) Black polypropylene netting to exclude the entry of debris

(B) Polyethylene cylinder that serves as the trap entrance and capture chamber

(C) Sticky surface made of a black styrene cylinder coated with a non-setting adhesive

(D) Screen barrier to prevent adult mosquitoes from reaching the infusion reservoir

(E) Black pail lid

(F) Black polyethylene pail,

(G) Drainage holes, (H) water, and (I) hay packet.

BG-Sentinel Traps

The most used indicators for vector surveillance

Larval surveys:

- *House index (HI)*: percentage of houses infested with larvae and/or pupae
- *Container index (CI)*: percentage of water-holding containers infested with larvae or pupae
- *Breteau index (BI)*: number of positive containers per 100 houses inspected
- **Pupae surveys:**
- *Pupa index (PI)*: number of pupae per 100 houses inspected
- **Adult surveys:**
- Estimating adult population density (abundance) using BG-sentinel traps, sticky traps, human landing collections or any similar traps = number of mosquitoes/trap/day

Examples of transmission thresholds associated with *Ae. aegypti*

Threshold	Disease	Location	Reference rate
Container Index (CI) < 10%	YF	South America	Connor and Monroe 1923
House Index (HI) < 5%	YF	South America	Soper 1967
Breteau Index (BI) < 5	YF	Senegal	Brown 1974
3 eggs/trap/day	DHF	Thailand	Mogi et al. 1990
0.5 – 1.5 pupae/person	DEN		Focks et al. 2000
0.5 females/trap/week (sticky traps)	DEN	Australia	Ritchie et al. 2004
3 mosquitoes/trap/week (AGO)	**CHIKV**	**PR**	**Barrera et al. 2017**
2 – 3 mosquitoes/trap/day (BG-Sentinel)	DEN	PR	Barrera et al. 2017

Resources

https://www.cdc.gov/zika/vector/range.html

https://www.cdc.gov/chikungunya/pdfs/Surveillance-and-Control-of-Aedes-aegypti-and-Aedes-albopictus-US.pdf

https://www.cdc.gov/zika/public-health-partners/vector-control-us.html

MosquitoNET

- Initial focus is on *Ae. aegypti* and *Ae. albopictus*
- Why only those species?
  - Vectors of several arboviruses of public health importance including CHIKV and ZIKV
  - Accurate distribution and abundance data lacking for these 2 species
- What about other mosquito species identified during surveillance?
  - Data on all other species collected during the course of the surveillance will be reported to MosquitoNET

Zika Forest

Zika – refers to overgrown
Ziika – refers to burying

Testing for Insecticide Resistance: The Bottle Bioassay

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"The findings and conclusions in this presentation are written by authors serving in their capacity as CDC employees and do not necessarily represent an official view of the Centers for Disease Control and Prevention."

CDC

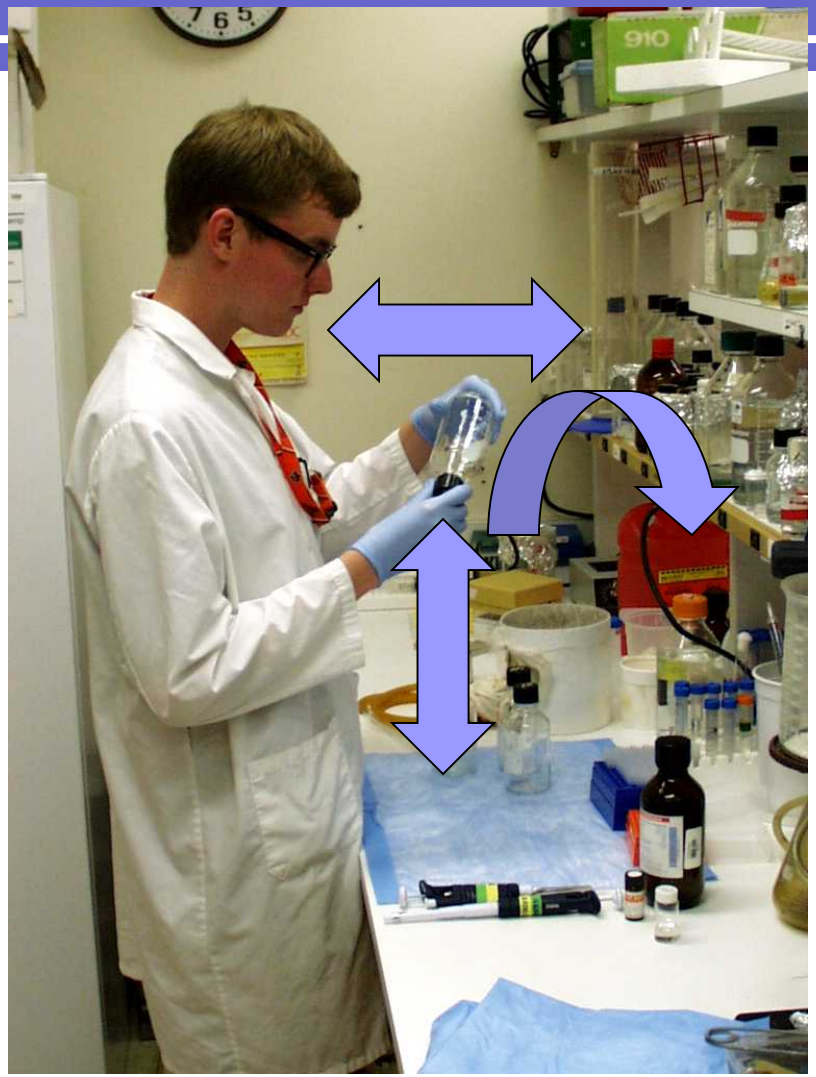
Testing Considerations

- There are multiple assays used to detect resistance.
- Assays do not correlate with operational control parameters. i.e. doses in assays ≠ label rates.
- Only caged field tests mimic operational control but are difficult to interpret unless done with susceptible mosquitoes to detect resistance.

Materials for Bottle Bioassay.

Add acetone and insecticide to bottle.

Coat bottle with insecticide.



Shake and roll in all directions

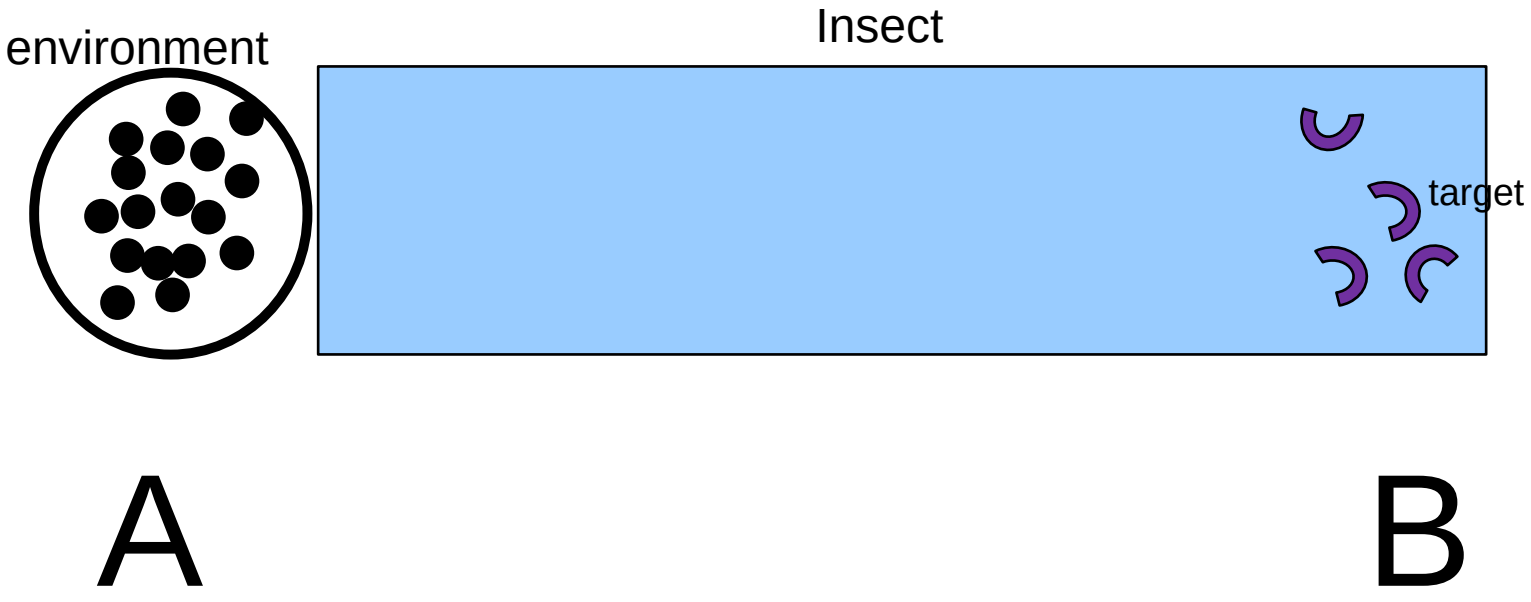
Evaporate acetone.

Roll bottles.

Adding Mosquitoes to Treated Bottles.

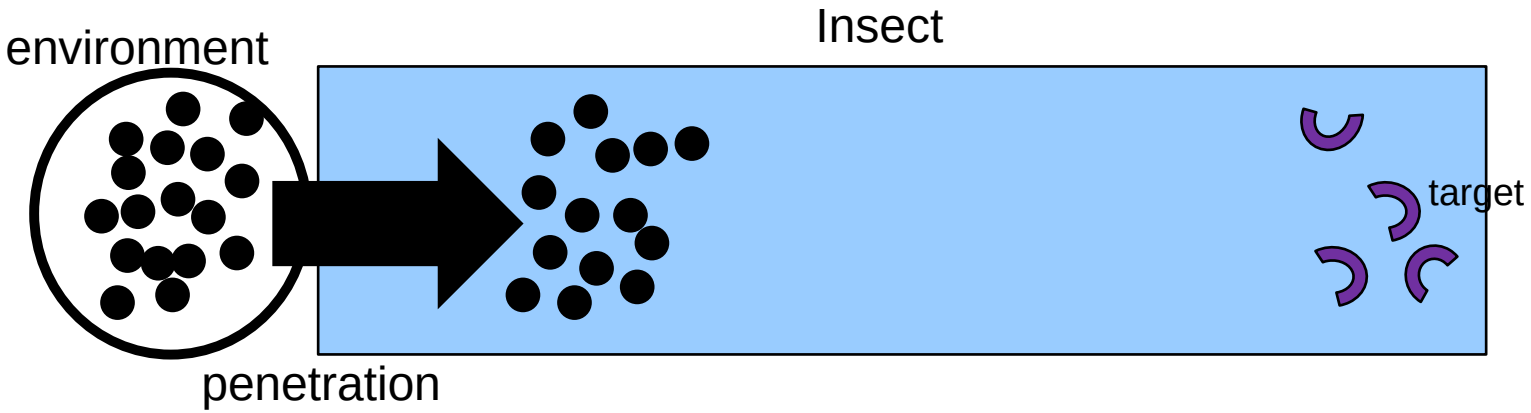
Reading Data.

Insecticide pathway



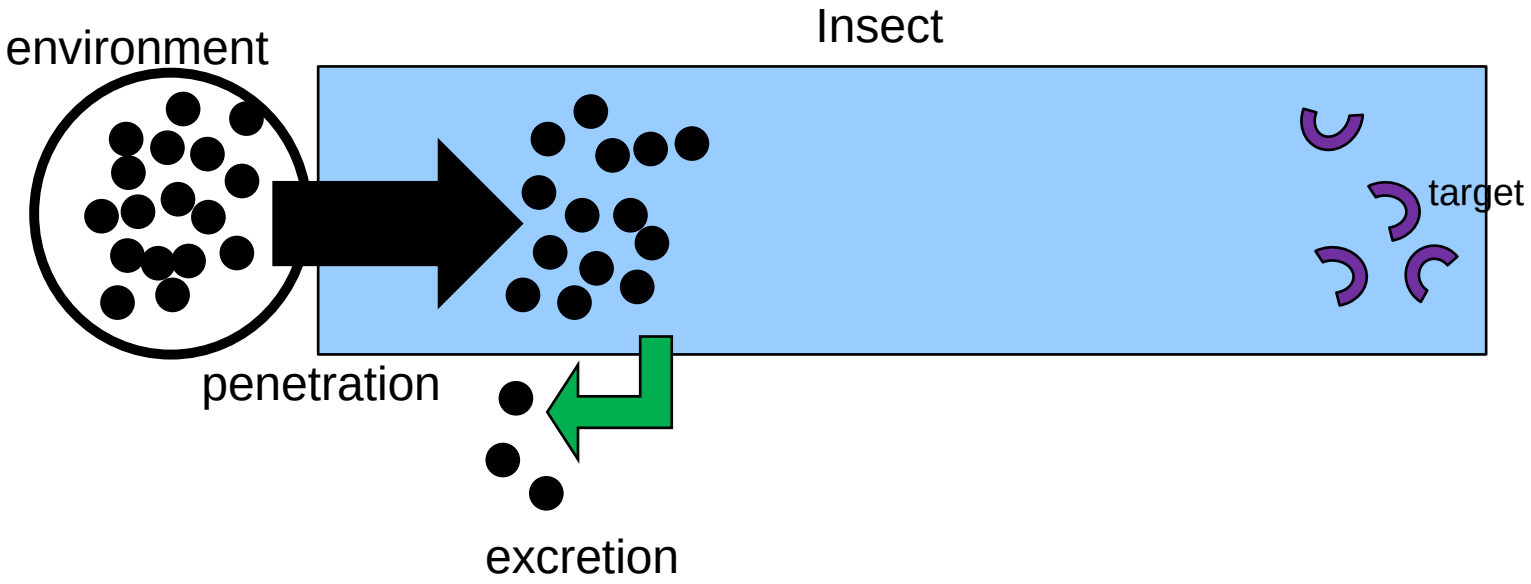
Susceptible insect

Insecticide pathway



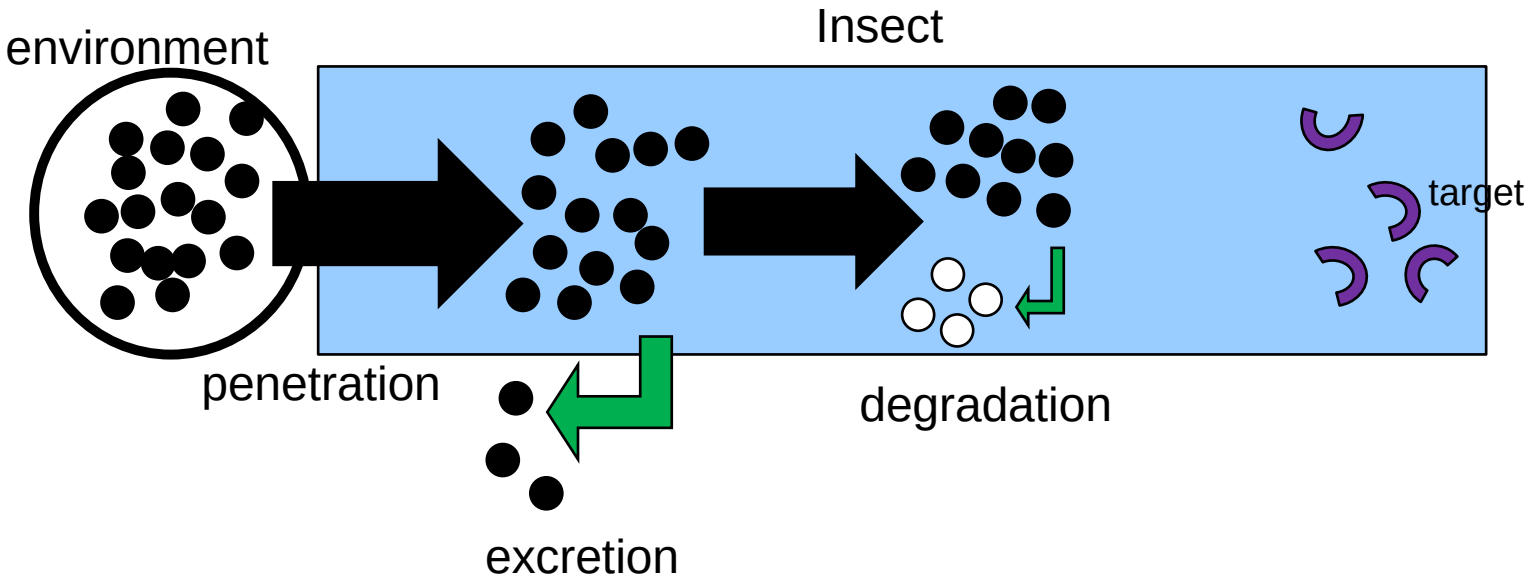
Susceptible insect

Insecticide pathway



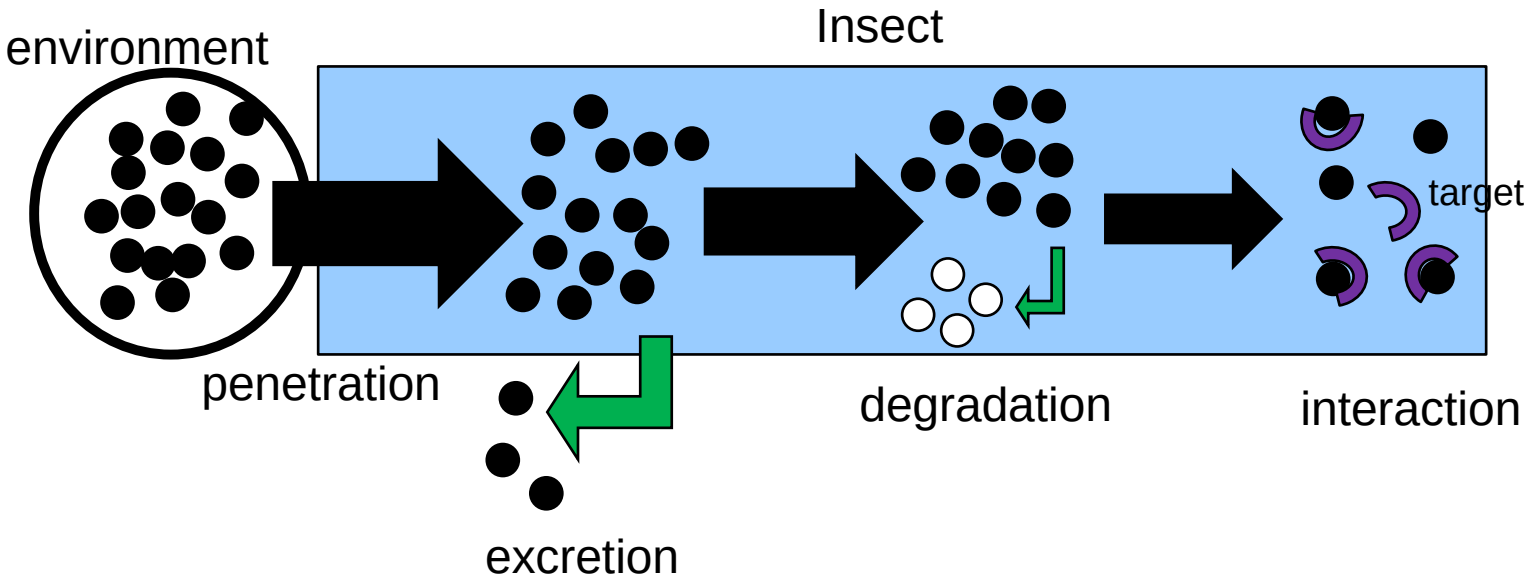
Susceptible insect

Insecticide pathway



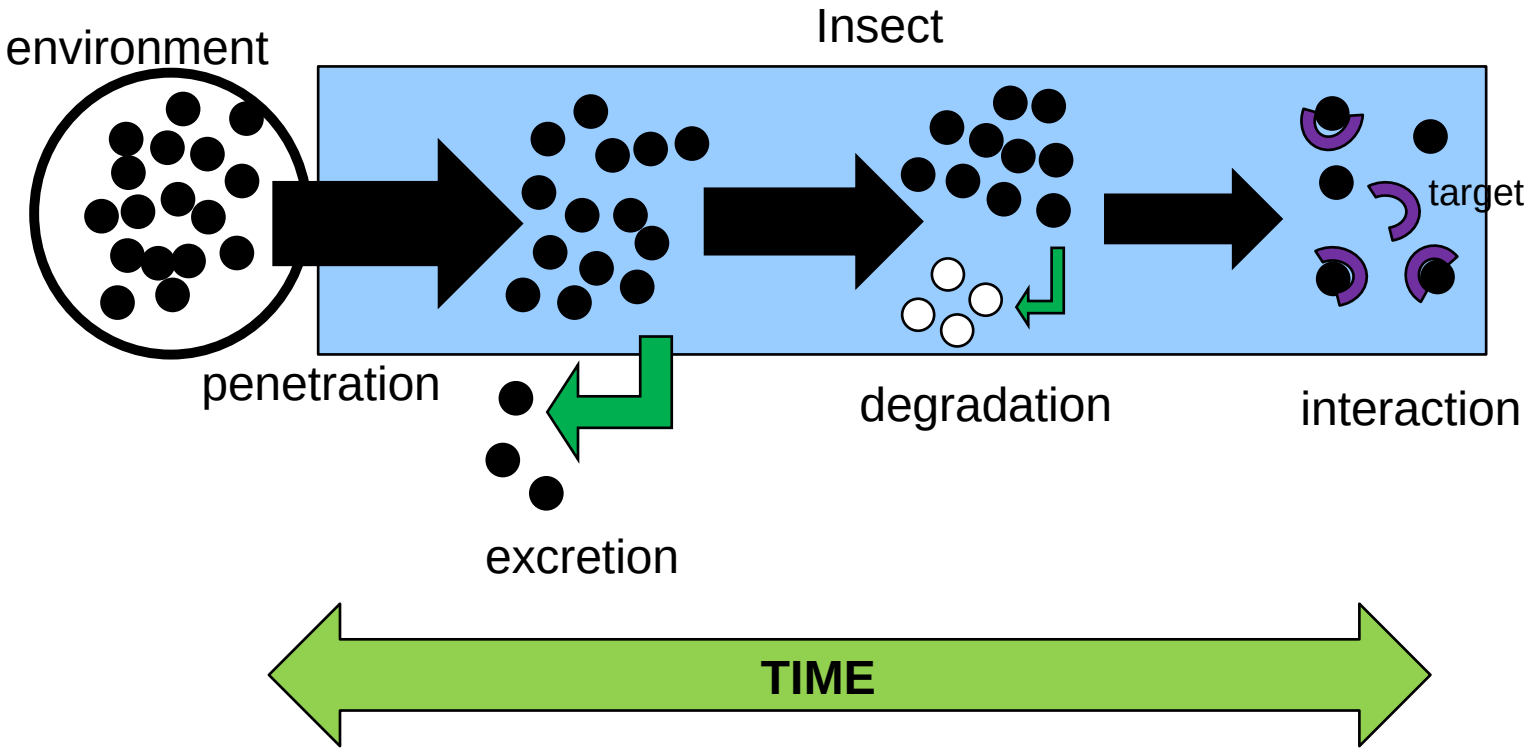
Susceptible insect

Insecticide pathway



Susceptible insect

Insecticide pathway



Susceptible insect

Chief Advantage of Bottle Bioassay

DIRECT MEASUREMENT OF THE CRITICAL TOXICOLOGICAL PARAMETER:

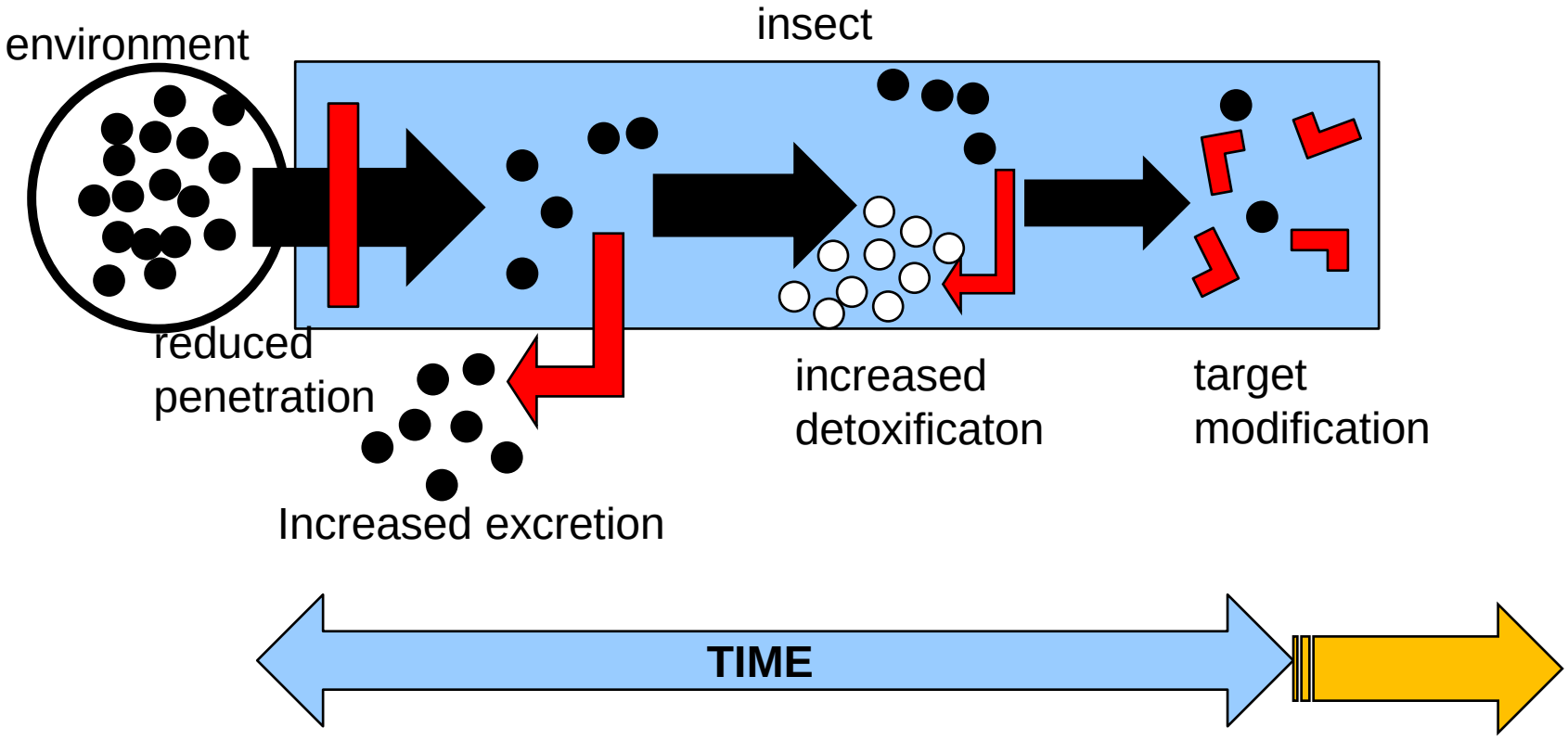
The length of time required for an insecticide to traverse intervening tissues to reach and interact with it's target in the presence or absence of any resistance mechanism(s).

Other Common Lab Tests

- WHO
- Dose Mortality (LD_{50}, LC_{50})
  - Topical Applications
  - Wind Tunnels

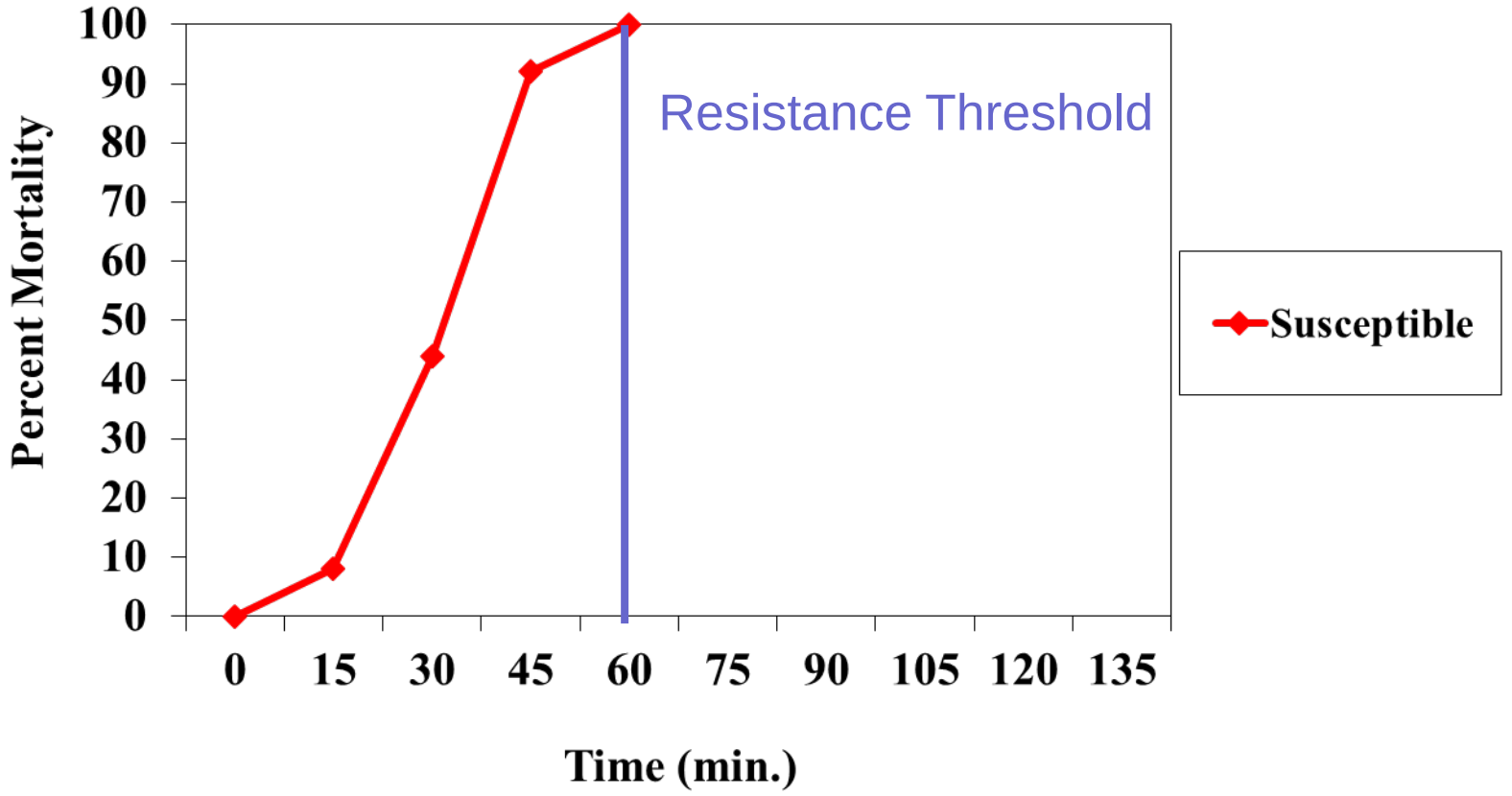
All are indirect measures of resistance and **must** be compared to a reference strain to generate resistance ratios for interpretation.

Insecticide pathway

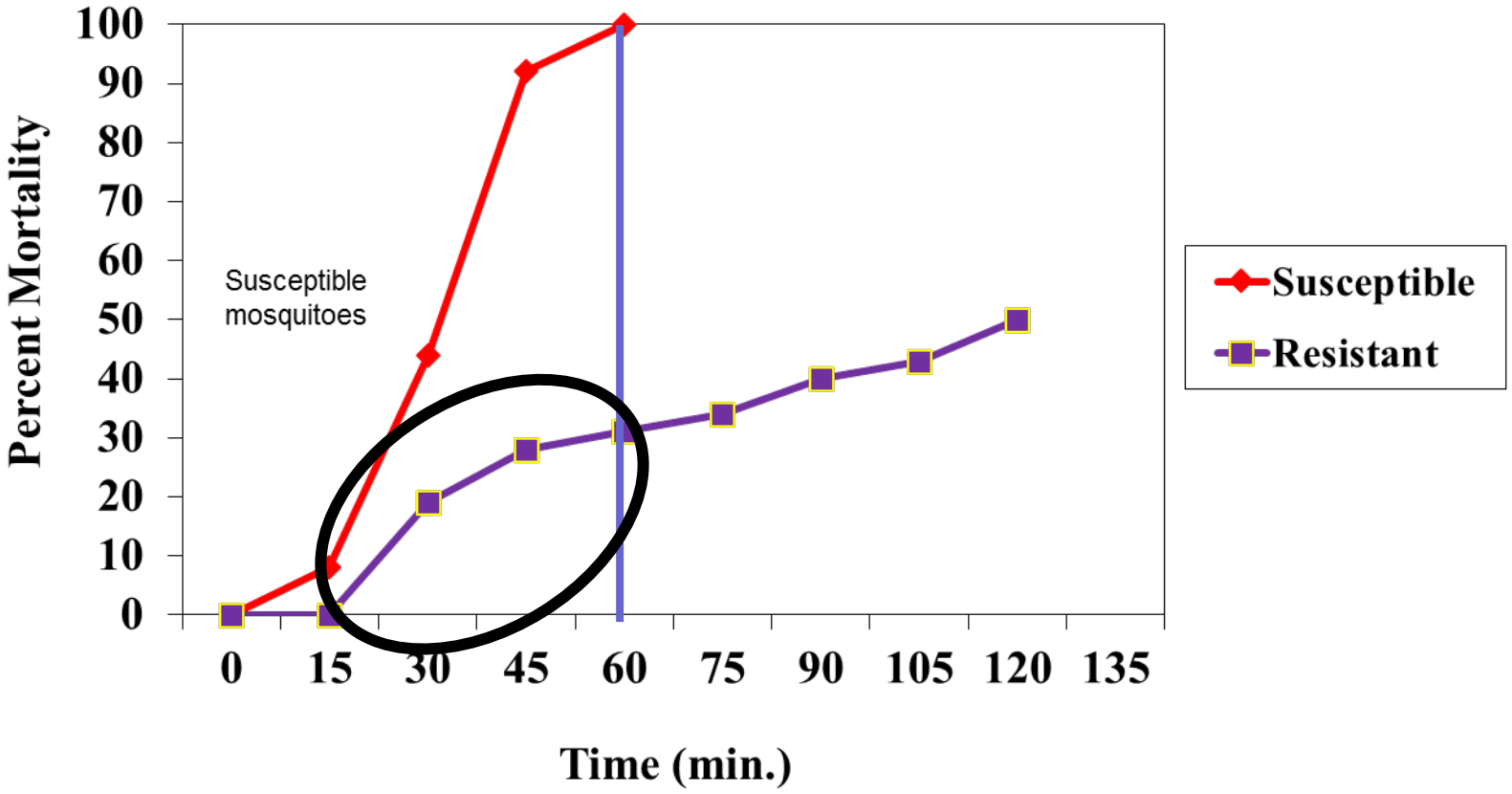


resistant insect

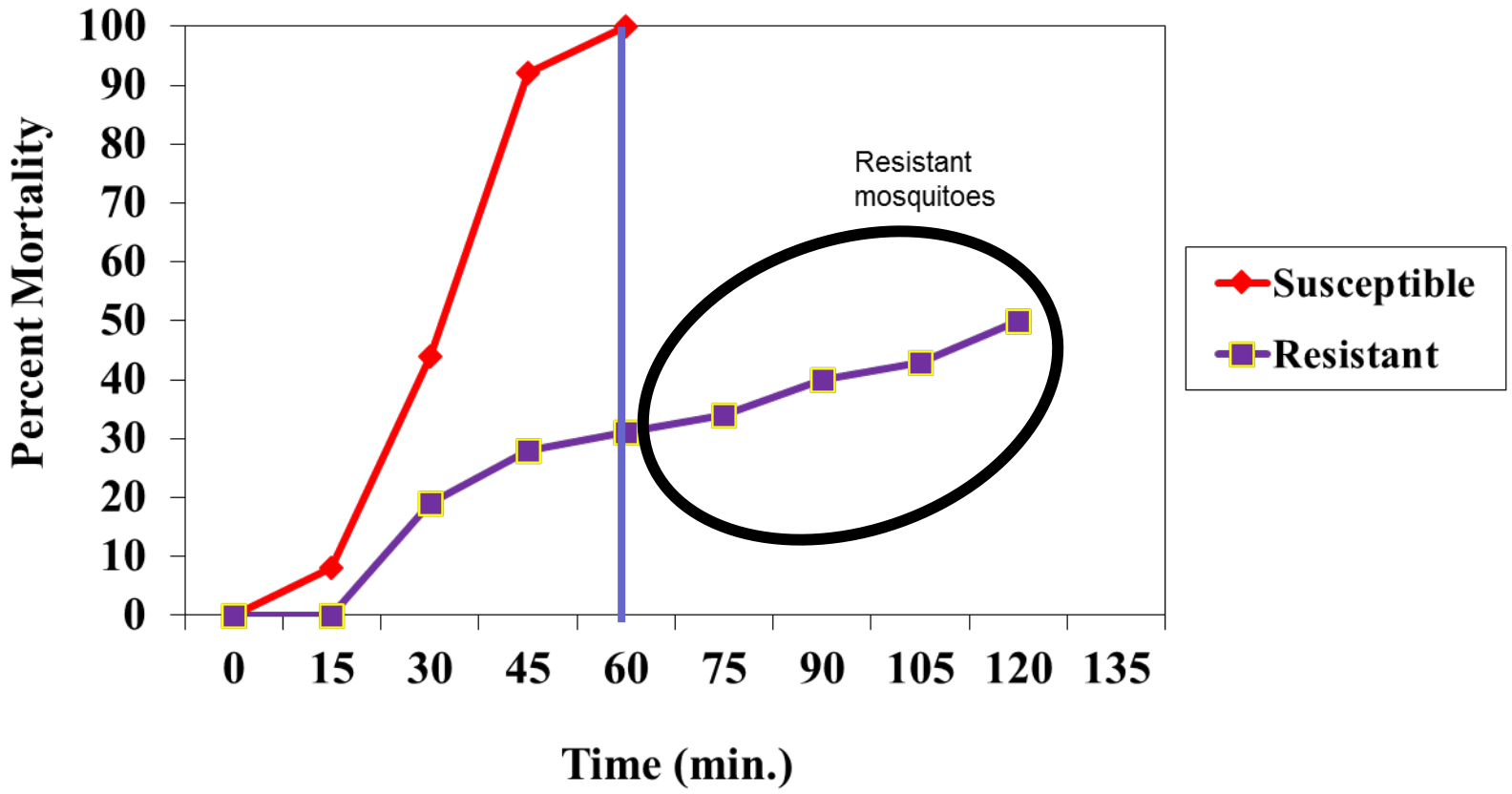
The upper range limit in minuets for survival of a representative susceptible population is selected as the resistance threshold.



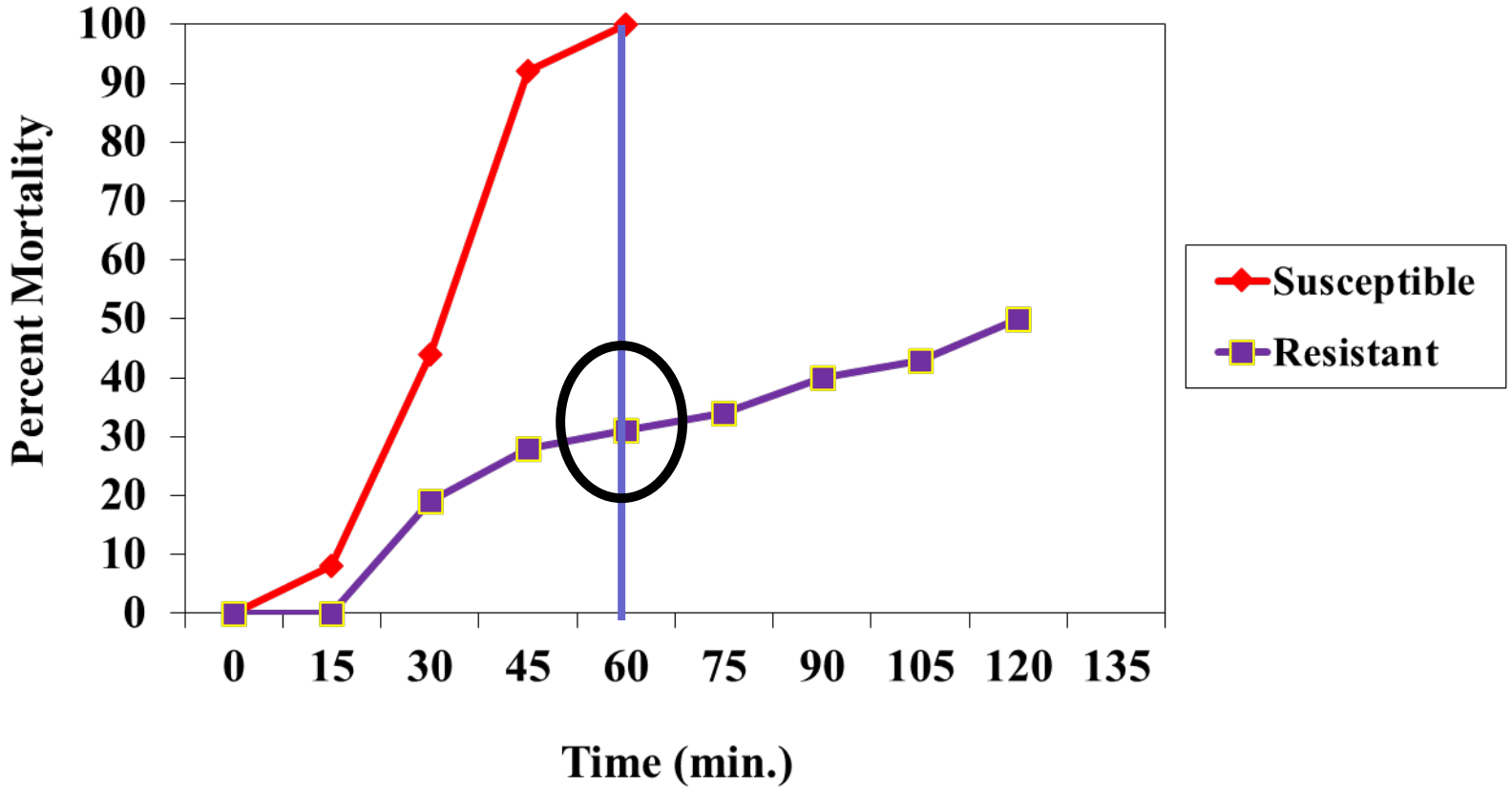
The proportion of the population to the left of the threshold time represent those that are susceptible



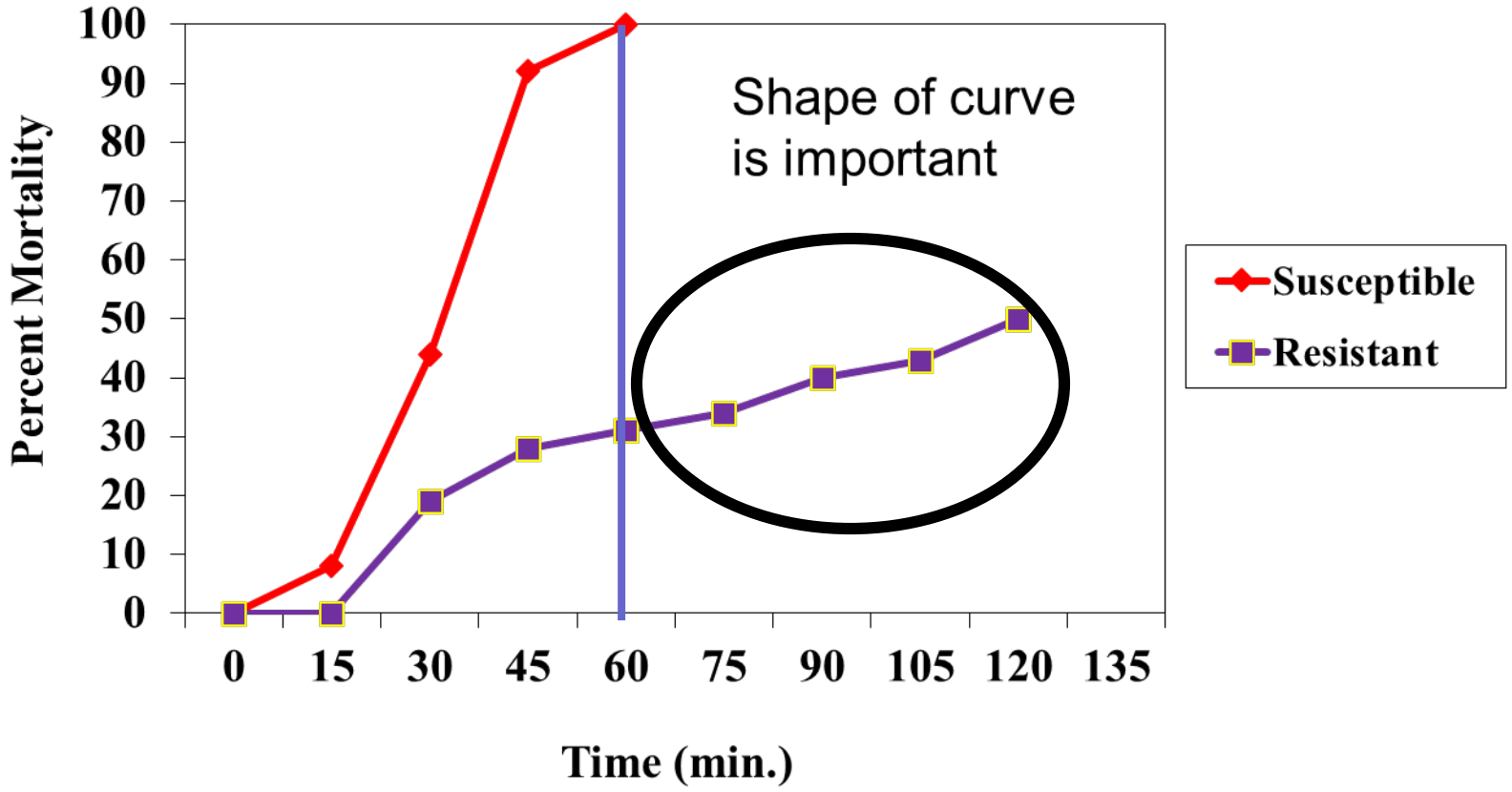
The mosquitoes that are to the right of the threshold time are resistant.



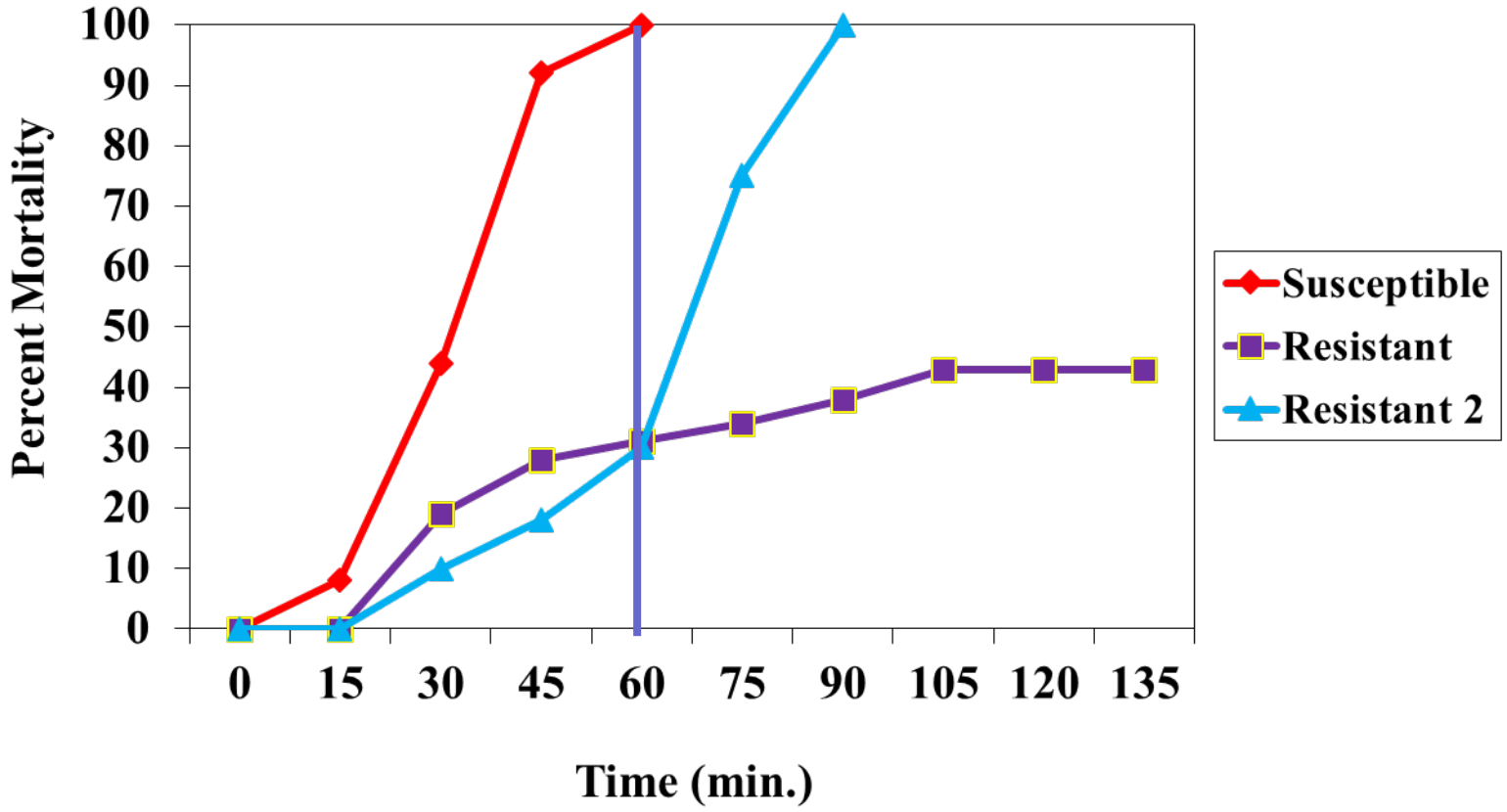
The percent mortality at the threshold time is the percent resistance in a population.



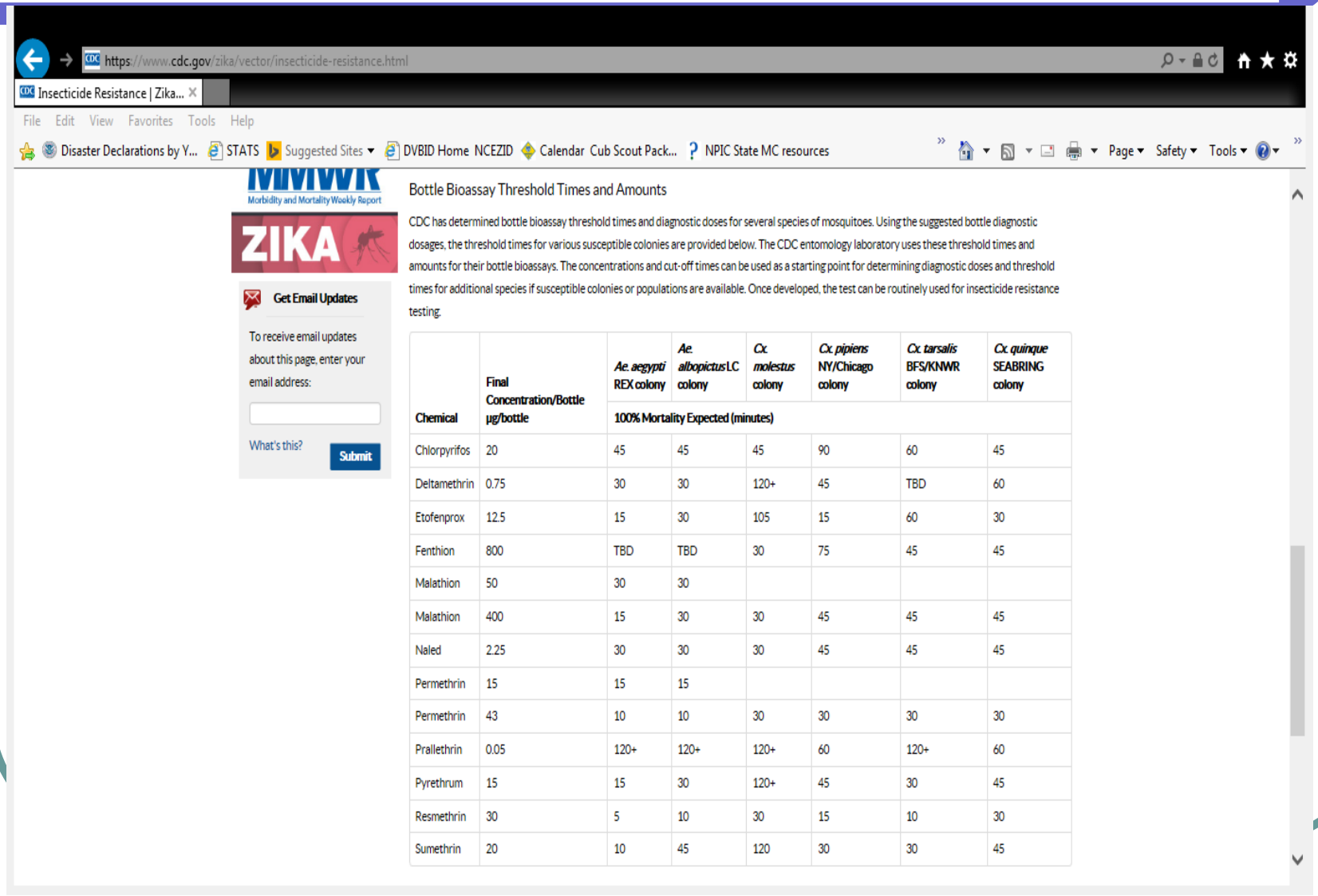
The share of the mortality curve is important, especially past the threshold time.



The strength of the resistance mechanism in indicated by what eventually happens to mosquitoes after the threshold time.



www.cdc.gov/zika



https://www.cdc.gov/zika/vector/insecticide-resistance.html

Insecticide Resistance | Zika...

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Disaster Declarations by Y... STATS Suggested Sites DVBID Home NCEZID Calendar Cub Scout Pack... NPIC State MC resources Page Safety Tools

MMWR Morbidity and Mortality Weekly Report

ZIKA

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Bottle Bioassay Threshold Times and Amounts

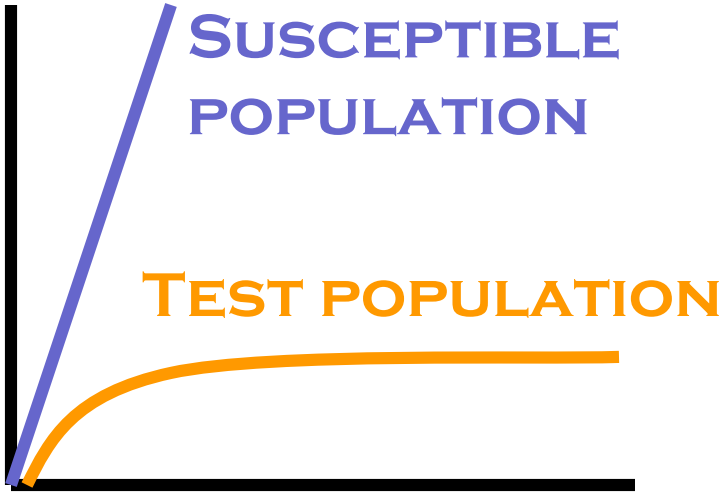
CDC has determined bottle bioassay threshold times and diagnostic doses for several species of mosquitoes. Using the suggested bottle diagnostic dosages, the threshold times for various susceptible colonies are provided below. The CDC entomology laboratory uses these threshold times and amounts for their bottle bioassays. The concentrations and cut-off times can be used as a starting point for determining diagnostic doses and threshold times for additional species if susceptible colonies or populations are available. Once developed, the test can be routinely used for insecticide resistance testing.

Chemical	Final Concentration/Bottle µg/bottle	*Ae. aegypti* REX colony	*Ae. albopictus* LC colony	*Cx. molestus* colony	*Cx. pipiens* NY/Chicago colony	*Cx. tarsalis* BFS/KNWR colony	*Cx. quinque* SEABRING colony
		100% Mortality Expected (minutes)					
Chlorpyrifos	20	45	45	45	90	60	45
Deltamethrin	0.75	30	30	120+	45	TBD	60
Etofenprox	12.5	15	30	105	15	60	30
Fenthion	800	TBD	TBD	30	75	45	45
Malathion	50	30	30				
Malathion	400	15	30	30	45	45	45
Naled	2.25	30	30	30	45	45	45
Permethrin	15	15	15				
Permethrin	43	10	10	30	30	30	30
Prallethrin	0.05	120+	120+	120+	60	120+	60
Pyrethrum	15	15	30	120+	45	30	45
Resmethrin	30	5	10	30	15	10	30
Sumethrin	20	10	45	120	30	30	45

Pyrethroid resistance bioassay.

Bottle treated with:

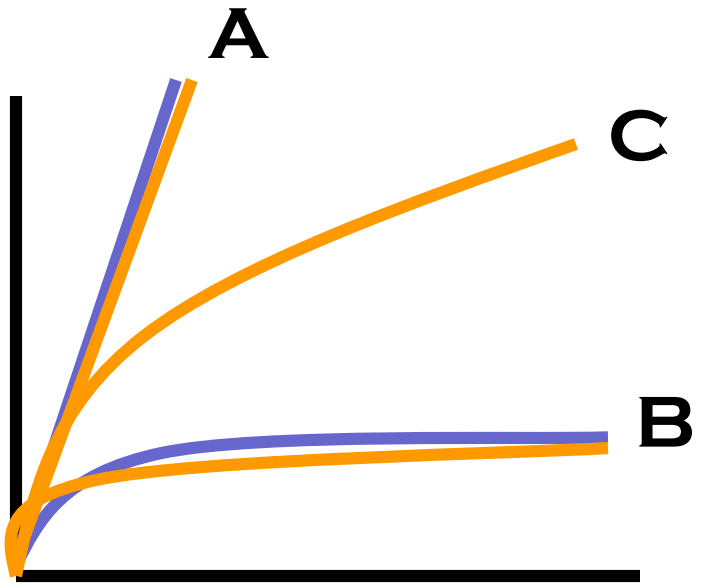
pyrethroid only



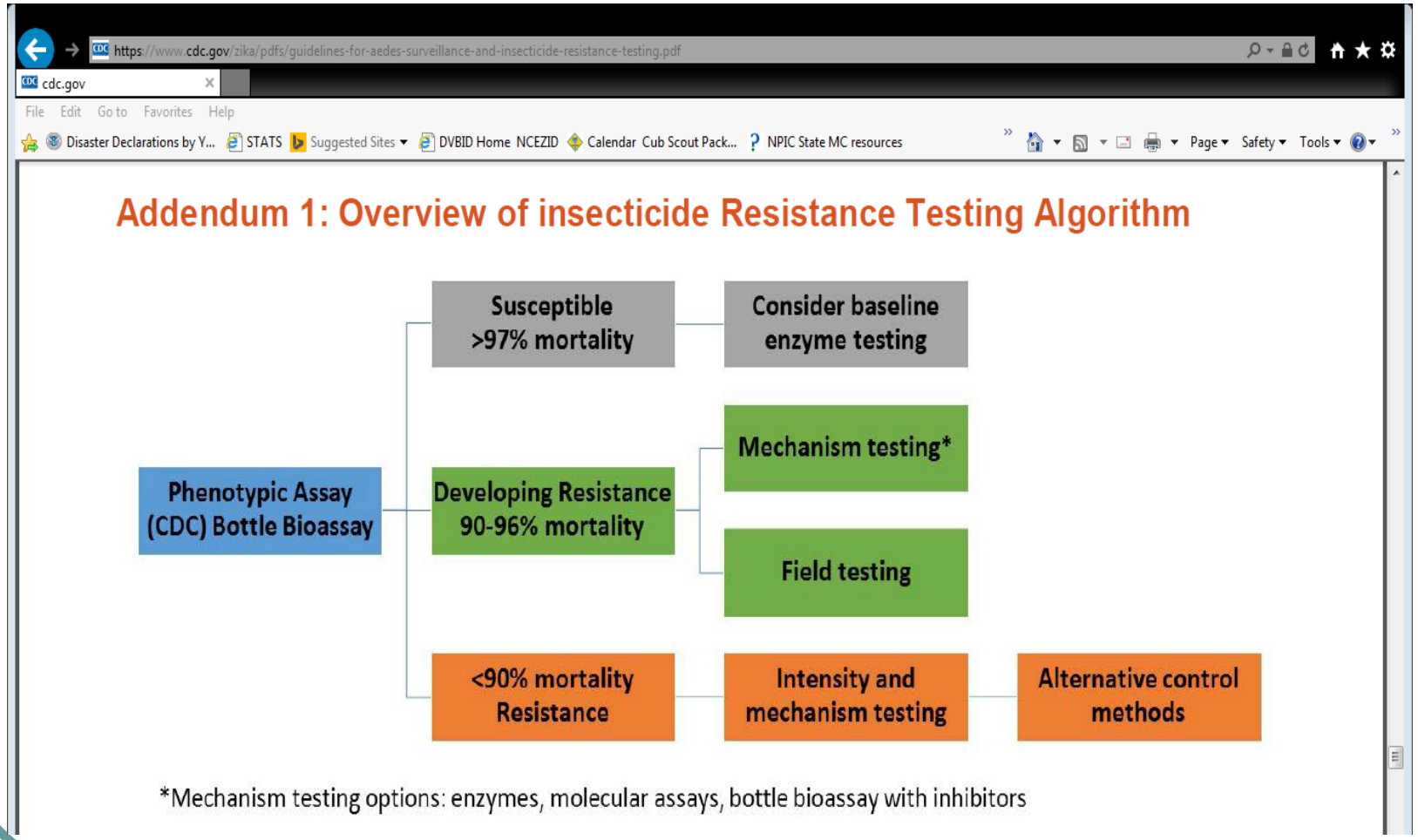
Oxidase resistance bioassay.

Bottle treated with:

pyrethroid + enzyme inhibitor



www.cdc.gov/zika

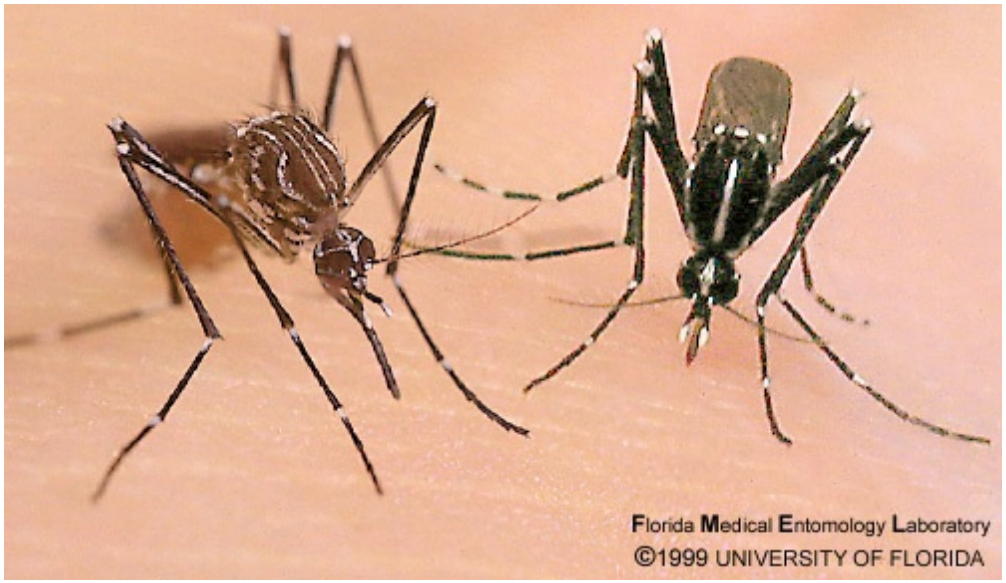


CDC Initiatives

- MosquitoNet
  - Platform to report resistance and surveillance data
  - Working on visualization of data
- Working on national resistance testing guidelines
- Developing IR test kit

jvm6@cdc.gov

Viral Surveillance for Zika: It is not all about the Six-Legged Bugs



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- consists of systematic collection of mosquito samples and screening them for arboviruses
- is the primary tool for quantifying the transmission of arboviruses and human risk
- is an integral component of an integrated vector management (IVM) program

Virus Surveillance in *Aedes aegypti* Populations

- Surveillance is conducted to:
  - Obtain evidence of local transmission
  - Estimate infection rates
  - Estimate local transmission thresholds
  - Evaluate the effectiveness of control measures
- Currently not emphasized by CDC:
  - Extremely difficult to detect virus in wild *Ae. aegypti* populations
  - Usually low mosquito abundance
  - Human/disease case surveillance is more efficient
  - Methods and equipment (traps) are improving

Am. J. Trop. Med. Hyg., 58(5), 1998, pp. 578–586

MONITORING OF DENGUE VIRUSES IN FIELD-CAUGHT *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* MOSQUITOES BY A TYPE-SPECIFIC POLYMERASE CHAIN REACTION AND CYCLE SEQUENCING

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Abstract. Virologic surveillance for dengue through the detection of the prevalent serotype(s) circulating in the human population during inter- and intra-epidemic periods constitutes a reliable sentinel system for dengue outbreaks. We have applied a rapid and sensitive, semi-nested, reverse transcription–polymerase chain reaction (RT-PCR) assay using nonstructural protein 3 gene primers for the type-specific-detection of dengue viruses in artificially infected and in field-caught adult *Aedes* mosquitoes. In laboratory experiments, the assay was sensitive enough to detect one virus-infected mosquito head in pools of up to 59 uninfected heads. In a prospective field study conducted from April 1995 to July 1996, female adult *Ae. aegypti* and *Ae. albopictus* mosquitoes were caught from selected dengue-sensitive areas in Singapore and assayed by RT-PCR. Approximately 20% of 309 mosquito pools were positive for dengue viruses. Of the 23 RT-PCR-positive *Ae. aegypti* pools (containing 1–17 mosquitoes each), 18 pools (78.3 %) were positive for dengue 1 virus. There were 40 RT-PCR-positive *Ae. albopictus* pools (containing 1–33 mosquitoes each) of which 31 (77.5%) were positive for dengue 1 virus. The predominant virus type responsible for the current dengue epidemic since 1995 was also dengue 1. The geographic locations of the virus-infected mosquitoes correlated with the residences or workplaces of patients within dengue outbreak areas. A total of 43.5% of the positive *Ae. aegypti* pools and 25.0% of the positive *Ae. albopictus* pools contained only a single mosquito. Both *Aedes* species showed similar overall minimum infection rates of 57.6 and 50 per 1,000 mosquitoes. Infected *Ae. aegypti* were detected as early as six weeks before the start of the dengue outbreaks in 1995 and 1996. However, infected *Ae. albopictus* appeared later, when the number of cases was increasing. Virologic surveillance by RT-PCR for detecting dengue virus–infected *Aedes* mosquitoes in the field may serve as an early warning monitoring system for dengue outbreaks.

Am. J. Trop. Med. Hyg., 74(4), 2006, pp. 678–683

HUMAN AND MOSQUITO INFECTIONS BY DENGUE VIRUSES DURING AND AFTER EPIDEMICS IN A DENGUE–ENDEMIC REGION OF COLOMBIA

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Abstract. We conducted a study in a dengue-endemic area of Colombia to evaluate the dynamics of transmission of dengue viruses during and after epidemics. Information was simultaneously gathered about occurrence of infection in humans and mosquitoes every three months in four cities with endemic transmission. Viral isolation was confirmed in 6.7% of the persons and most were asymptomatic. Adult mosquito and larvae house indexes were not found associated with increased burden of disease. The only entomologic indicator related to dengue infection in humans was the pooled infection rate of mosquitoes. *Aedes aegypti* infection rates showed significant differences between the epidemic (10.68, 95% confidence interval [CI] = 7.04–15.62) and after epidemic periods of the study (6.15, 95% CI = 3.46–10.19). In addition, *Ae. albopictus* were also infected with dengue viruses. Increases in mosquito infection rates were associated with increases in human infection rates in the following trimester.

Mendez *et al.* 2006

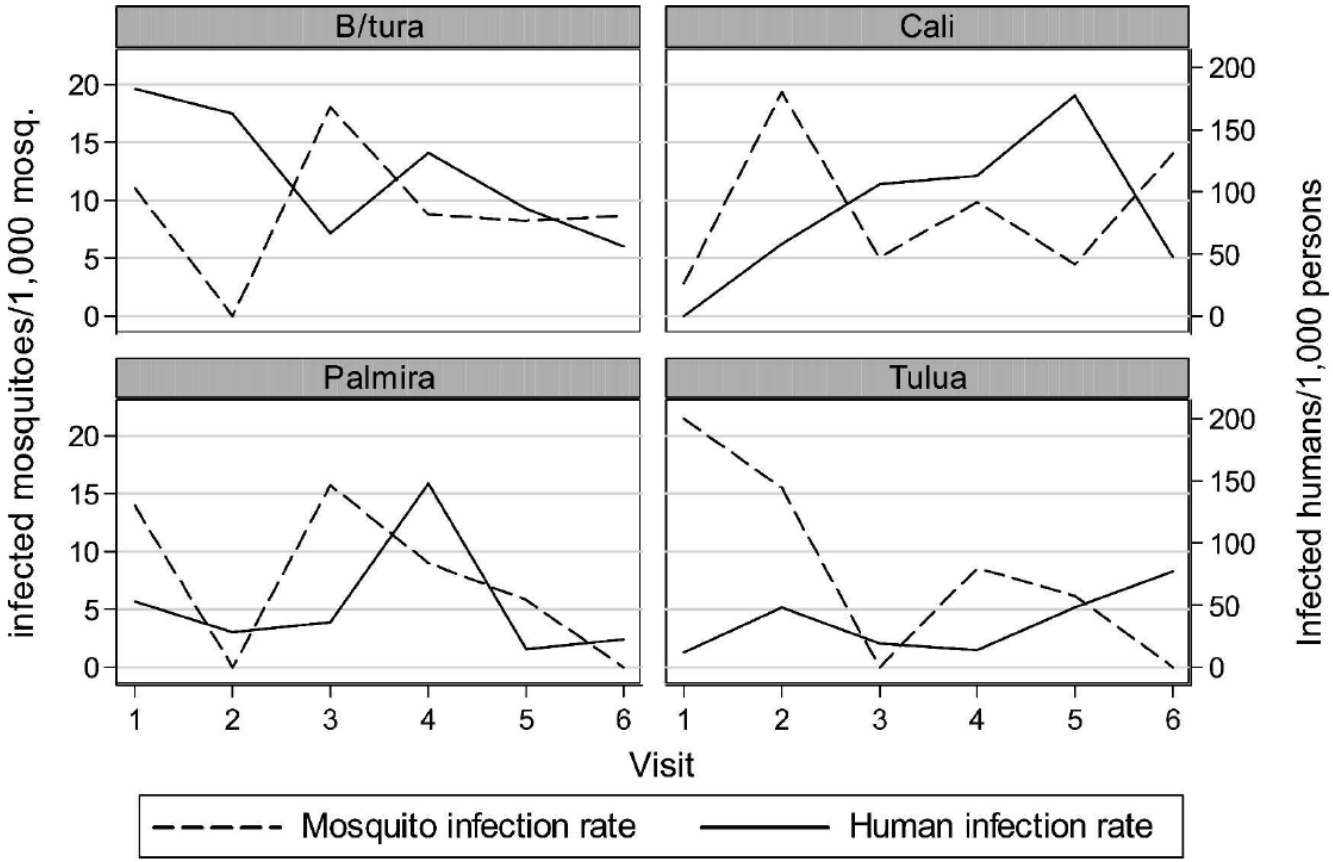


FIGURE 4. Pooled infection rate in mosquitoes (mosq.) and dengue-virus isolation rate in humans by city and visit (every three months) in four cities in Valle de Cauca, Colombia, November 2002–March 2004. B/tura = Buenaventura.

Screening of Dengue Virus in Field-Caught *Aedes egypti* and *Aedes albopictus* (Diptera: Culicidae) by One-Step SYBR Green-Based Reverse Transcriptase-Polymerase Chain Reaction Assay During 2004–2007 in Southern Taiwan

Chen *et al.* 2010

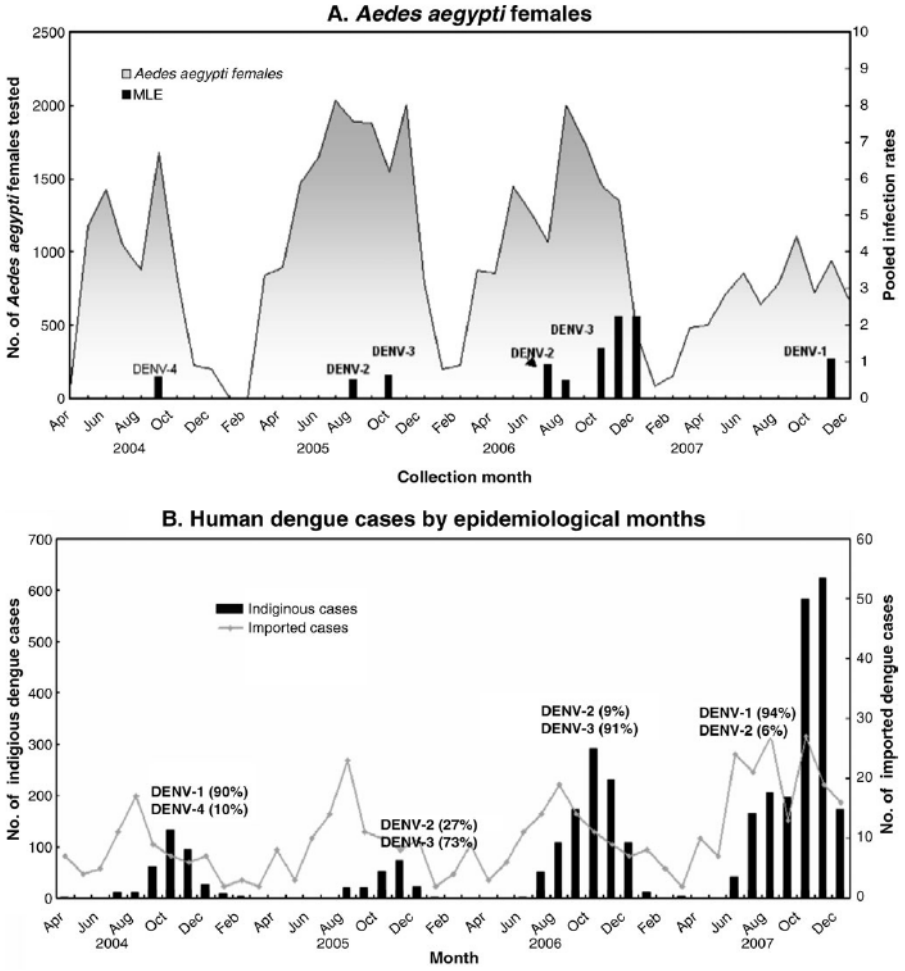


FIG. 2. Number of mosquitoes tested and dengue virus (DENV) infection rates in *Ae. aegypti* females (A) and human dengue cases of dengue (B) from 2004 to 2007 in southern Taiwan.

Collecting Resting *Aedes aegypti* Indoors

BG-Sentinel Traps

Virus Surveillance in *Aedes aegypti* Populations

- Detecting virus-infected mosquitoes before an outbreak involves sampling large numbers of mosquitoes
- *Ae. aegypti* usually exists in low relative abundance – may involve sampling a large number of sites
- Monitoring virus in mosquito populations is the best way of predicting where outbreaks are likely to occur and/or where subclinical infections are taking place
- Urban areas frequented by large numbers of travelers and established *Ae. aegypti* populations should be primary targets for continuous monitoring

Mosquito Processing

- All efforts should be made to transport mosquitoes alive or in a cool container to maximize the chances of keeping the virus viable
- Field-collected mosquitoes must be sorted and identified on cold surfaces (chill table) to maximize the chances of detecting virus
- The identified mosquitoes are pooled into groups of 50 or less mosquitoes for arbovirus testing
- The different species, sexes and trap locations are pooled separately to keep track of arboviral infections in different species and arboviral infestation at different locations
- If screening is not done right after mosquito identification, the pooled samples should be stored at -70^{O}C

Laboratory Screening

Real-Time RT-PCR	–	quick
	-	specific
Cell Culture	-	detection many viral agents
	-	virus isolation

Mosquito-based surveillance indicators

Vector Index

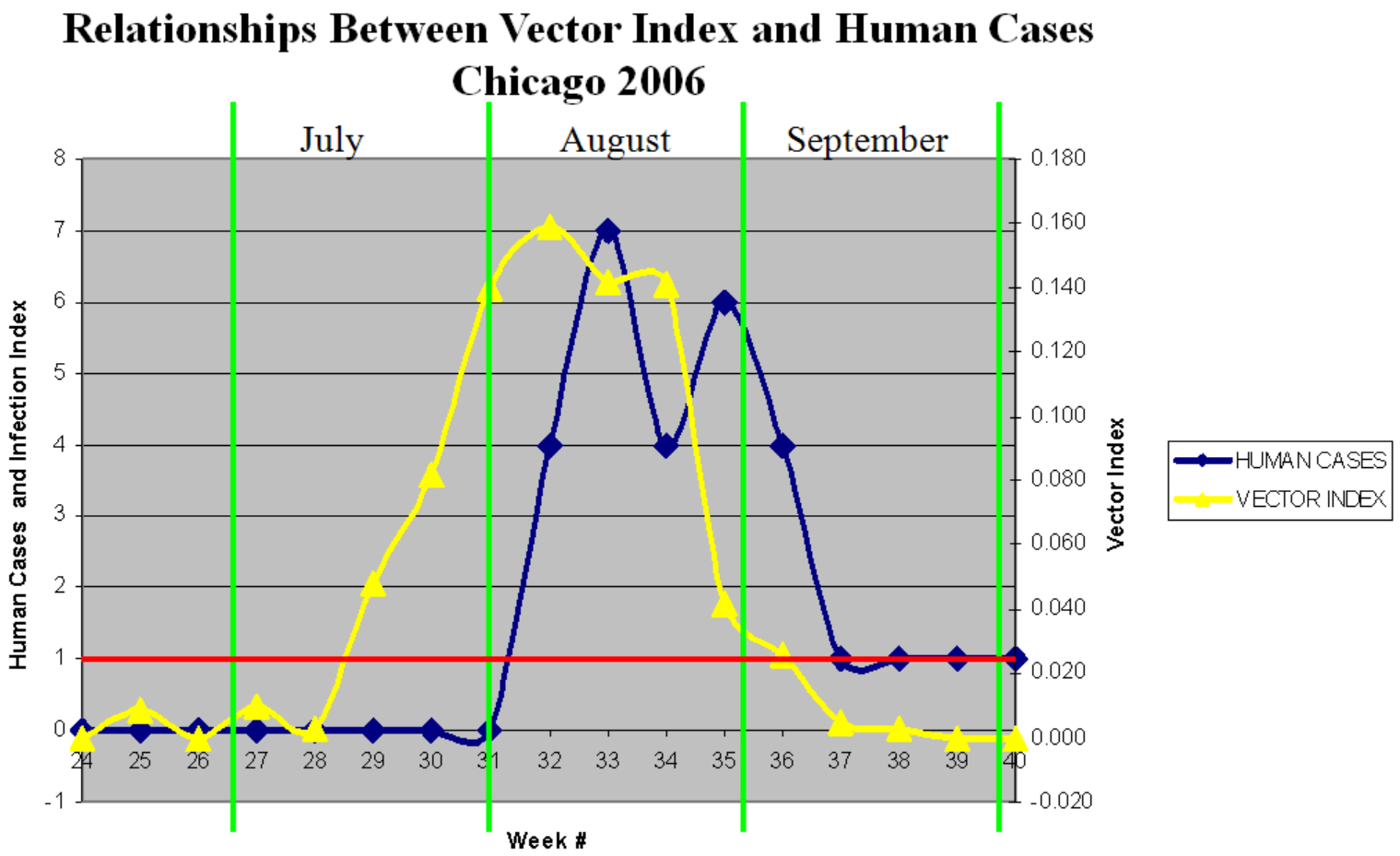
Advantages

- Provides indicator of the abundance of infected mosquitoes in an area (VI = proportion infected x number collected per trap night)
- Accommodates multiple vector species in an area
- Permits variable pool number and size

Limitations

- Sample size dependent (more specimens tested = better estimate of infected vector abundance)
- Consistent procedures and effort required for comparability over time and space

*If you are going collect and test mosquitoes, use Vector Index


Relationships Between Vector Index and Human Cases
Chicago 2006
July
August
September
Human Cases and Infection Index
Vector Index
Week #
HUMAN CASES
VECTOR INDEX
8
7
6
5
4
3
2
1
0
-1
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
-0.020
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Zika Tower: Uganda Constructed in 1961

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