Insecticide Resistance in Mosquitoes

Stephanie Richards[1], Jo Anne Balanay[1], Avian V. White[1], Joe Hope[2], Kurt Vandock[2]

[1]Environmental Health Science Program
Department of Health Education and Promotion
East Carolina University

[2]Bayer Crop Science
Environmental Science, Vector Control and Pest Management

East Carolina UNIVERSITY

Introduction

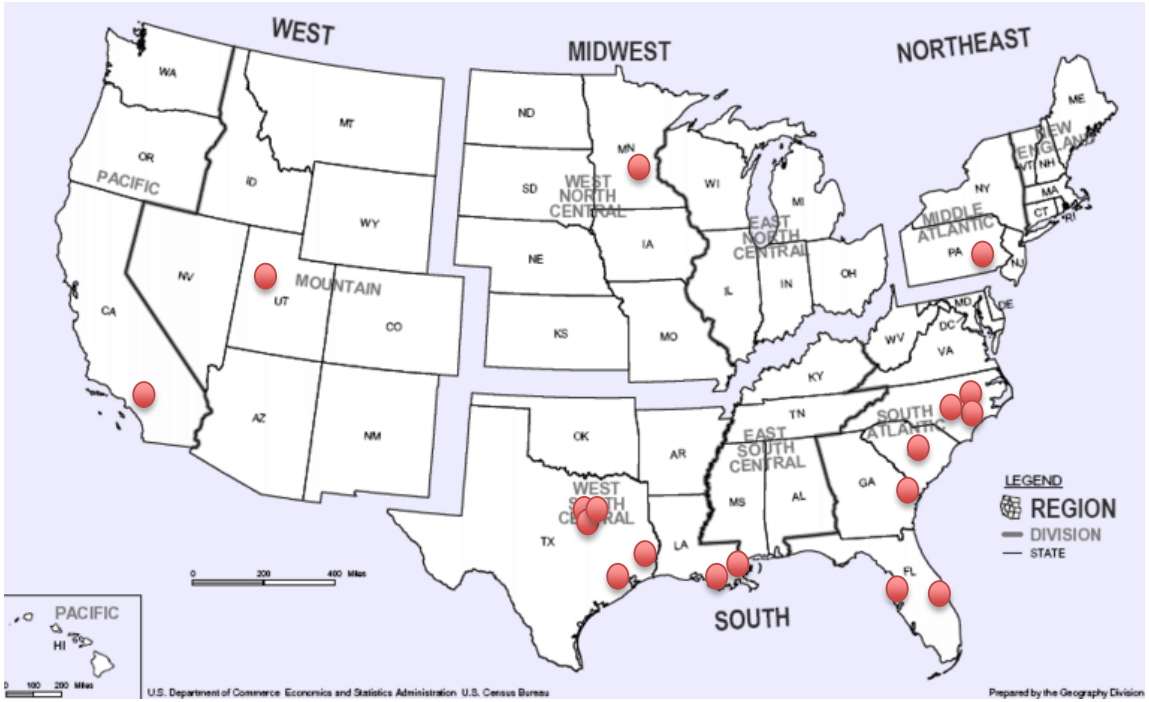
- Routine mosquito susceptibility/resistance monitoring is an important part of a successful mosquito control program.
  - Informs management decisions by informing selection of the most effective active ingredients used in control measures.

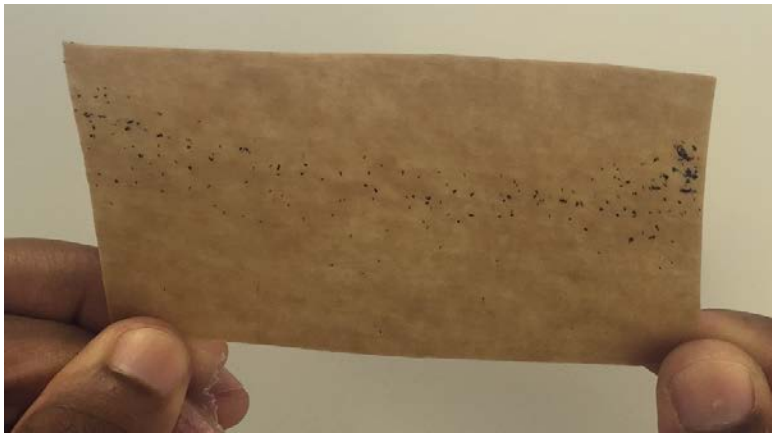
Study Objectives

- Determine susceptibility/resistance for several US mosquito populations against six active ingredients commonly used in mosquito control.
- Investigate the extent to which susceptibility/resistance differs between active ingredients, mosquito collection locations, and (for some populations) years.

Study Design

- 17 mosquito populations were collected by abatement districts, control programs, and/or universities from 4 US regions and eggs were mailed to investigators.
- Approximately 5 more populations still to be tested.





Study Design

- Eggs of *Aedes albopictus*, *Ae. aegypti*, *Culex nigripalpus*, *Cx. pipiens*, *Cx. pipiens/quinquefasciatus*, and *Cx. quinquefasciatus* were reared to adults.
- Generation F_0 utilized when possible.
- If necessary, mosquitoes were blood fed and additional generations were propagated to increase sample size.

Study Design

- Six active ingredients tested (technical grade).
- CDC bottle bioassays.
- Doses standardized across mosquito populations for comparison.
- 30 min diagnostic time used for all except malathion (60 min)
  - Malathion (100 µg/mL* and 250 µg/mL**)
  - Etofenprox (6 µg/mL* and 15 µg/mL**)
  - Permethrin (15 µg/mL**)
  - Bifenthrin (12.6 µg/mL**)
  - Deltamethrin (5 µg/mL** and 10 µg/mL*)
  - Phenothrin (23 µg/mL**)

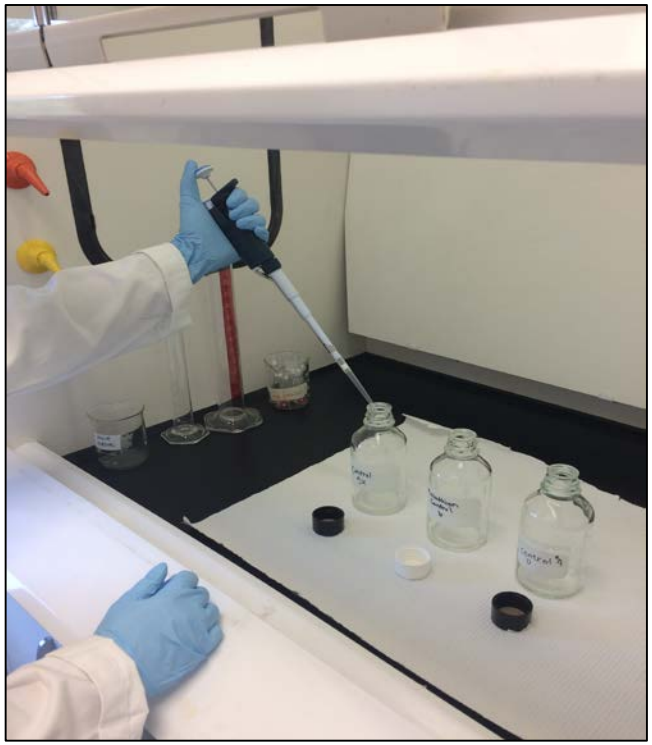
*doses used in 2015 and based on dose-response curves for susceptible *Aedes albopictus* colony in 2015

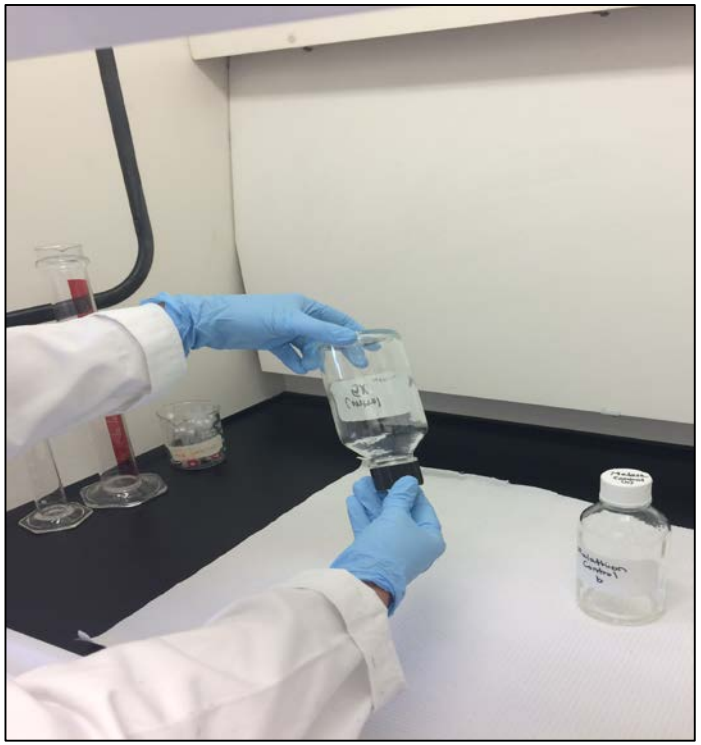
**doses based on dose-response curves for susceptible colonies (*Culex quinquefasciatus* and *Aedes albopictus*) conducted in 2016

Study Design

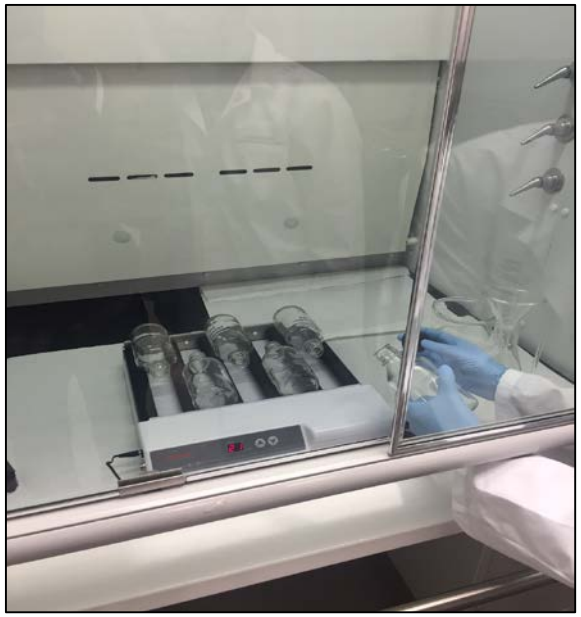
- Active ingredient standards prepared in acetone.
- Solutions stored at 4°C for duration of experiment.
- Concentrations verified every 2 weeks to test for degradation of active ingredients.
  - Analyzed 3 - 4 replicate samples (1 µL) / stock solution.
  - Capillary gas chromatograph with flame ionization detector.
  - Calibration curves generated for quantification.

Methods





- 1 mL of each active ingredient stock/bottle.
- Inside of bottles coated.
- At least 3 control bottles (1 mL acetone) used in each assay.



- Caps removed and bottles placed on bottle roller until contents evaporated (1 - 2 minutes)
- Uncapped bottles placed into dark drawer and used within 24 h.

- Mosquitoes transferred to bottles with mechanical aspirator.
- CDC bottle bioassays conducted for all active ingredients.

Appendix 3. CDC bottle bioassay data recording form

Date: _____ Mosquito species: _____

Insecticide: _____

Diagnostic dose: _____ Diagnostic time: _____

Location of mosquito collection: _____

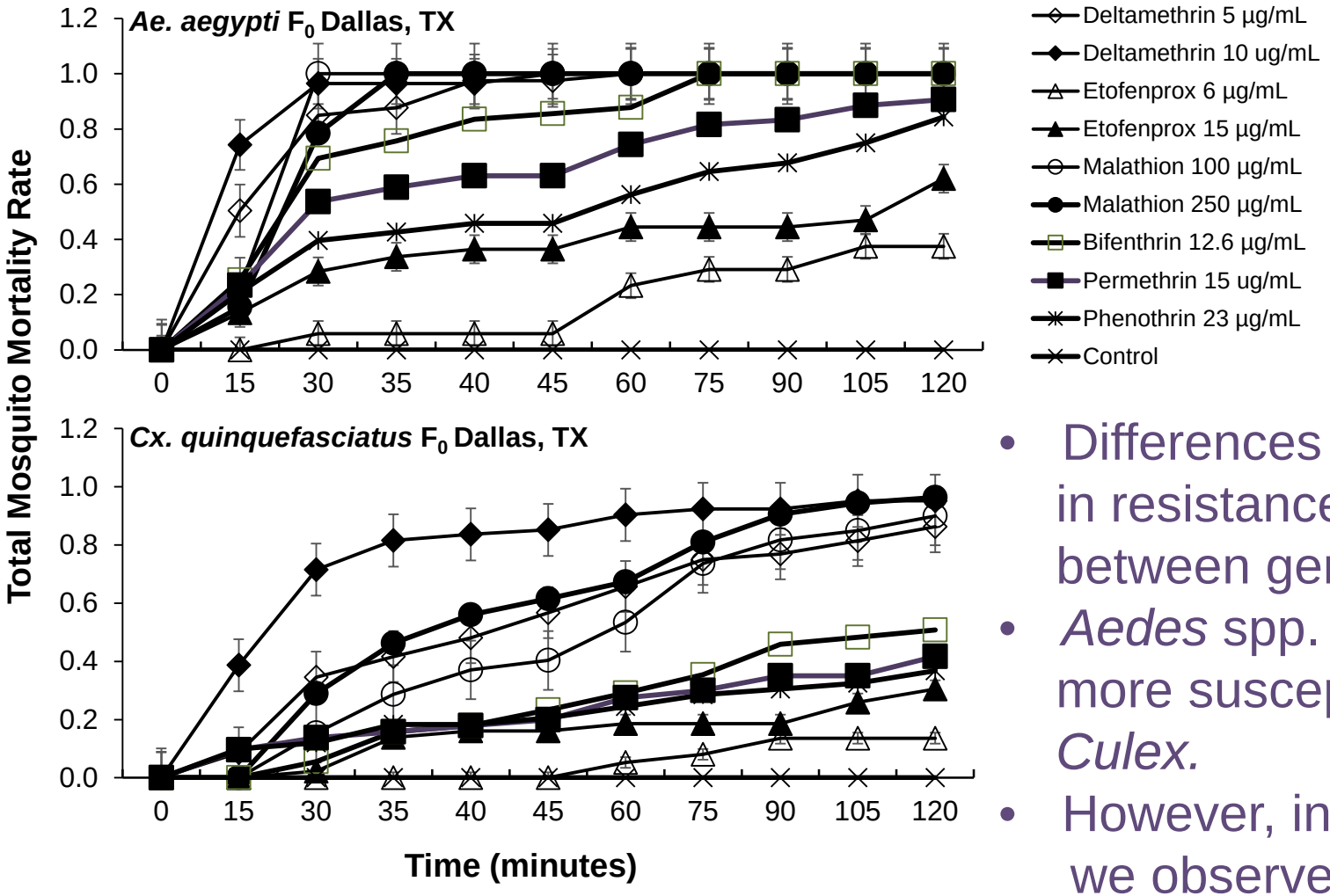
Time (min)	Bottle 1		Bottle 2		Bottle 3		Bottle 4		All test bottles			Control		
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Total dead	Total	% dead	Total dead	Total	% dead
0														
15														
30														
35														
40														
45														
60														
75														
90														
105														
120														
Total in bottle														

- Mosquito mortality recorded at 11 time points using the “CDC bottle bioassay data recording form”.

Data Analyses

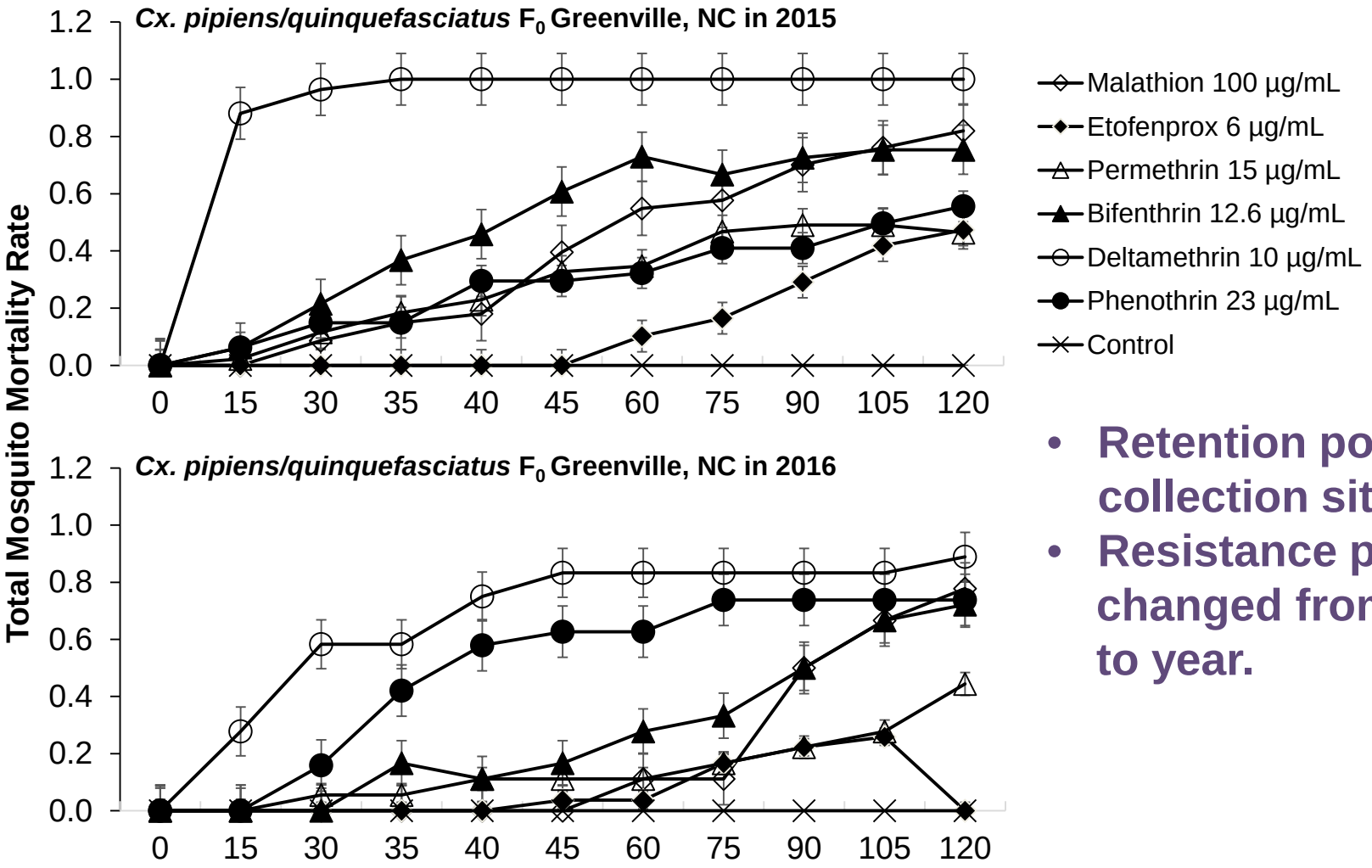
- Ordinal logistic regression ($P < 0.05$) used to show differences in susceptibility, possible resistance, and resistance, if any, between active ingredients.
- World Health Organization guidelines:
  - Susceptible: ≥ 98% mortality at the diagnostic time
  - Possible resistance: 80-97% mortality
  - Resistance: < 80% mortality
- Odds ratios computed to illustrate differences between active ingredients.

Examples of Results – South



- Differences observed in resistance profiles between genera**.**
- *Aedes* spp. generally more susceptible than *Culex.*
- However, in this case, we observed resistance in *Aedes* for several active ingredients.

Examples of Results – Comparing One Collection Site Between 2015 and 2016



- **Retention pond collection site.**
- **Resistance profiles changed from year to year.**

Results Summary

- Highest to lowest average (%) mortality at diagnostic time

  Purple = susceptible Orange = possible resistance Black = resistant

- *Ae. albopictus* (N = 4 populations)

  Permethrin = Phenothrin ≈ Deltamethrin = Etofenprox > Deltamethrin > Bifenthrin > Malathion > Malathion > Etofenprox

  (100) (100) (98)(10 µg/mL) (98)(15 µg/mL) (95)(5 µg/mL) (92) (89)(250µg/mL) (67)(100µg/mL) (52)(6µg/mL)

- *Ae. aegypti* (N = 1 population)

  Malathion = Malathion > Deltamethrin ≈ Deltamethrin > Bifenthrin ≈ Permethrin ≈ Phenothrin ≈ Etofenprox ≈ Etofenprox

  (100)(250µg/mL)(100)(100µg/mL) (96)(10 µg/mL) (95)(5 µg/mL) (69) (54) (40) (28)(15 µg/mL) (6)(6µg/mL)

- *Cx. nigripalpus* (N = 1)

  Deltamethrin > Permethrin ≈ Deltamethrin > Phenothrin = Bifenthrin > Etofenprox > Malathion > Etofenprox > Malathion

  (75)(10 µg/mL) (61) (59)(5 µg/mL) (37) (37) (31)(15 µg/mL) (13)(250 µg/mL) (4)(6 µg/mL) (0)(100 µg/mL)

- *Cx. pipiens* (N = 3)

  Deltamethrin > Deltamethrin > Malathion ≈ Phenothrin > Bifenthrin ≈ Malathion > Permethrin > Etofenprox > Etofenprox

  (99)(10 µg/mL) (90)(5 µg/mL) (72)(250 µg/mL) (71) (62) (61)(100 µg/mL) (45) (27)(15 µg/mL) (0)(6 µg/mL)

- *Cx. quinquefasciatus* (N = 6)

  Deltamethrin > Malathion > Deltamethrin > Malathion > Bifenthrin ≈ Phenothrin ≈ Permethrin > Etofenprox > Etofenprox

  (63)(10 µg/mL) (37)(250 µg/mL) (34)(5 µg/mL) (24)(250 µg/mL) (17) (16) (14) (8)(15 µg/mL) (2)(6 µg/mL)

- *Cx. pipiens/quinquefasciatus* (N = 2)

  Malathion > Deltamethrin > Deltamethrin > Phenothrin > Etofenprox ≈ Etofenprox ≈ Bifenthrin > Malathion ≈ Permethrin

  (75)(250 µg/mL) (72)(10 µg/mL) (57)(5 µg/mL) (44) (39)(15 µg/mL) (37)(6 µg/mL) (36) (26)(100 µg/mL) (24)

General Observations

- *Aedes* spp. and *Culex* spp. exhibited variation in resistance/susceptibility.
- Resistance to active ingredients was 15 times higher in *Culex* compared to *Aedes*.

General Observations

- Some mosquito populations were highly resistant (never achieved 80% mortality for duration of experiment).
- No *Culex* species were classified as “susceptible” for malathion (100 or 250 µg/mL), etofenprox (6 or 15 µg/mL), bifenthrin, or permethrin.
- No *Aedes* species were classified as “susceptible” for etofenprox (6 µg/mL).
- Resistance profiles varied between years.

Discussion

- Mosquito control programs may enact different insecticide pressures on *Culex* (ULV applications at dusk/dawn) compared to *Aedes* (residual barrier sprays).
- Other sources of insecticide pressure
  - e.g., agricultural, homeowner applications
- This study evaluated technical grade active ingredients and not formulated products that may contain synergists and/or other ingredients.

Discussion

- We expect variation in susceptibility and/or resistance of other mosquito species from different regions, for other active ingredients, and for these populations from year to year.
- Routine surveillance of insecticide resistance is important and enhances the ability of control programs to protect public health.
- Only the most effective insecticides should be used for targeted control.

Acknowledgements

- This research was supported by Bayer Crop Science.
- We thank the many mosquito control programs and universities that contributed mosquitoes used in the study.