Characterization of insecticide resistance in *Culex pipiens* from the lower Chesapeake Bay

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Reasons for Resistance Monitoring

- Stewardship
- Suspected control failures
- Tracking changes in resistance over time
- Provides data that can help agencies make operational decisions

Considerations

- Field efficacy of formulated products is helpful
- We do not test formulated products

Formulated products present problems when interpreting the CDC bottle bioassay

A water based dual-action Adulticide for Effective Control of Adult Mosquitoes, Gnats, Non-biting Midges, Biting and Non-biting Flies in Outdoor Residential and Recreational Areas.

ACTIVE INGREDIENTS:	
Prallethrin: (RS)-2-methyl-4-oxo-3-(2-propynyl) cyclopent-2-enyl-(1RS)-cis,trans-chrysanthemate	1.00%
Sumithrin®: 3-Phenoxybenzyl-(1RS, 3RS,1RS, 3SR)-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecarboxylate	5.00%
* Piperonyl Butoxide	5.00%
OTHER INGREDIENTS	89.00%
	100.00%

Contains 0.084 pounds of Prallethrin/Gallon, 0.422 pounds of Sumithrin®/Gallon and 0.422 pounds of Technical Piperonyl Butoxide/Gallon

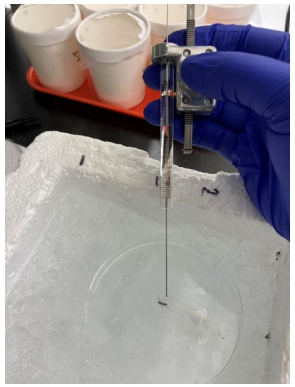
*(butylcarbityl)(6-propylpiperonyl) ether and related compounds

CDC Bottle Bioassay

- Sensitive to the presence of resistance
- Limited steps
- Time based assay
- Imprecise

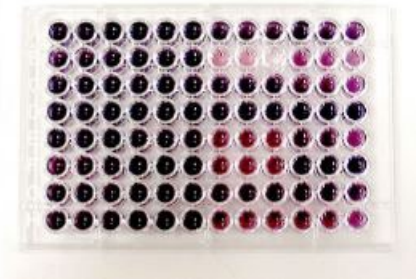
Topical Assay

- Determines lethal concentrations
- Precise
- Known dosage based on mosquito size
- Time consuming



Microplate Assay

- Metabolic mechanisms of resistance
- Precise
- Does not indicate level of resistance



Submission System

Culex pipiens
&
Aedes albopictus

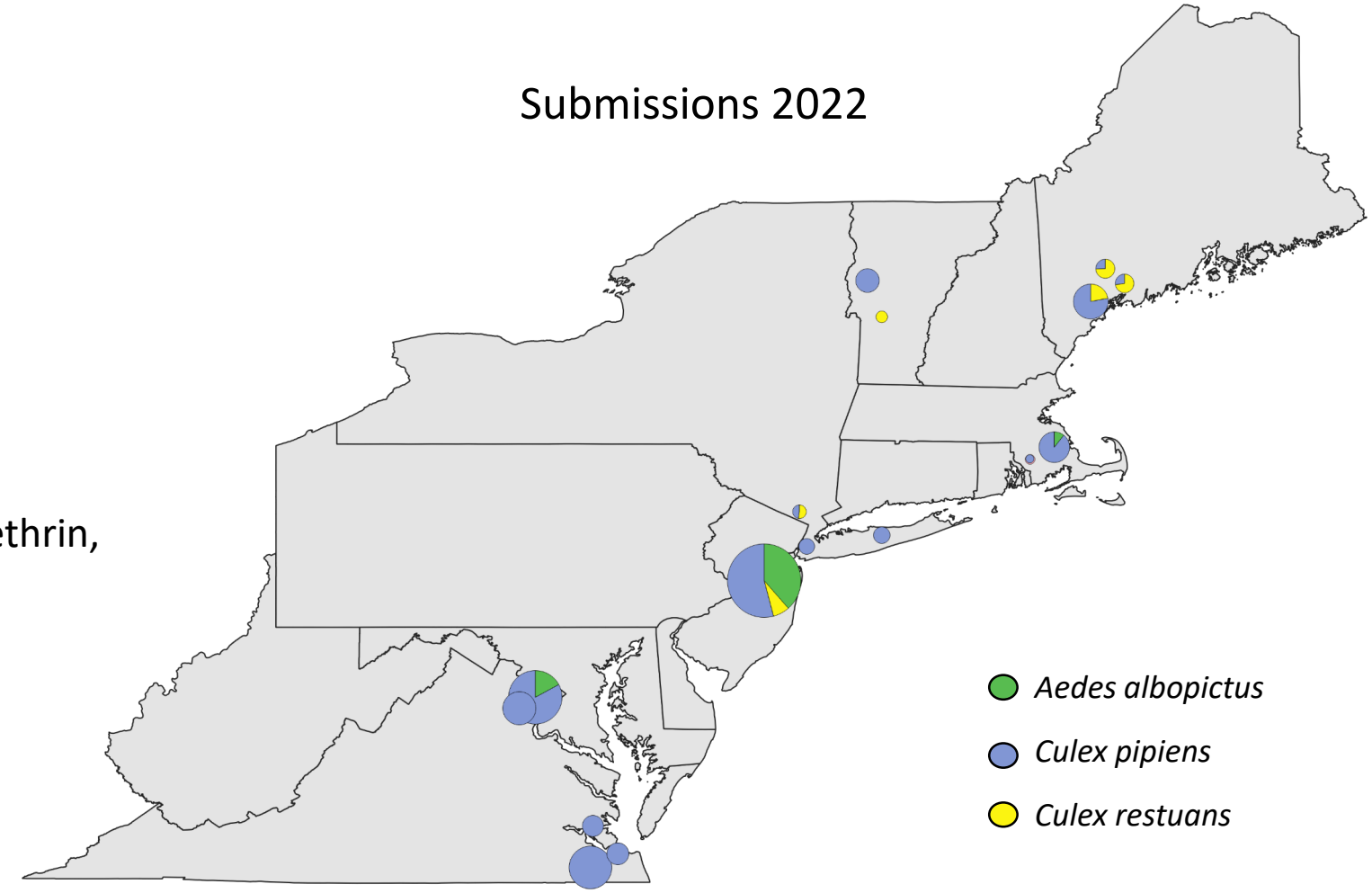
Larval assays (cup assay)

- *Bacillus thuringiensis israelensis* (Bti)
- *Lysinibacillus sphaericus* (Ls)
- Methoprene (Juvenile hormone analog)

Adult assays (CDC bottle bioassay)

- Pyrethroids: Sumithrin, Pyrethrum, Prallethrin, Permethrin, Deltamethrin
- Organophosphates: Chlorpyrifos, Naled, Malathion

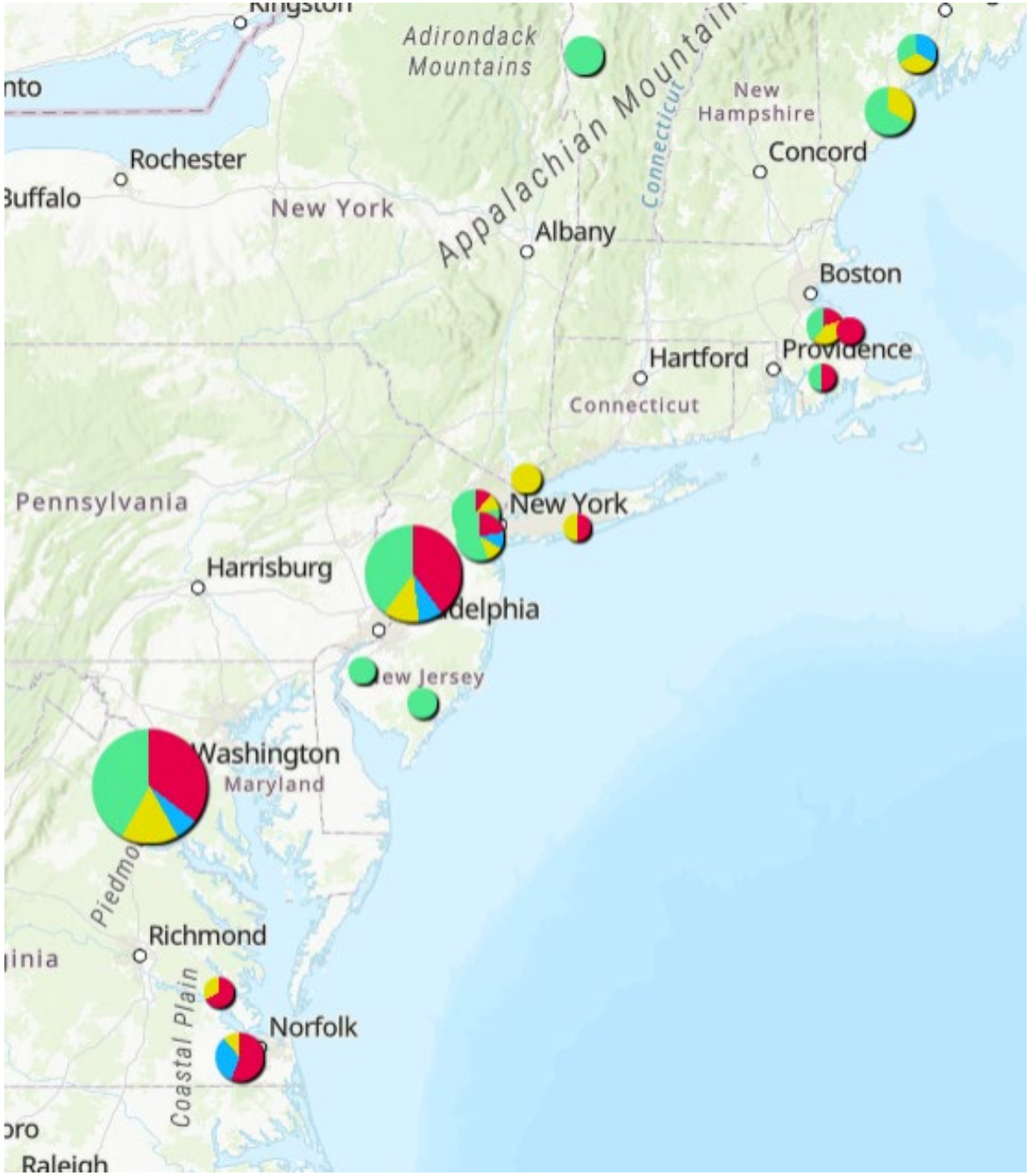
Submissions 2022



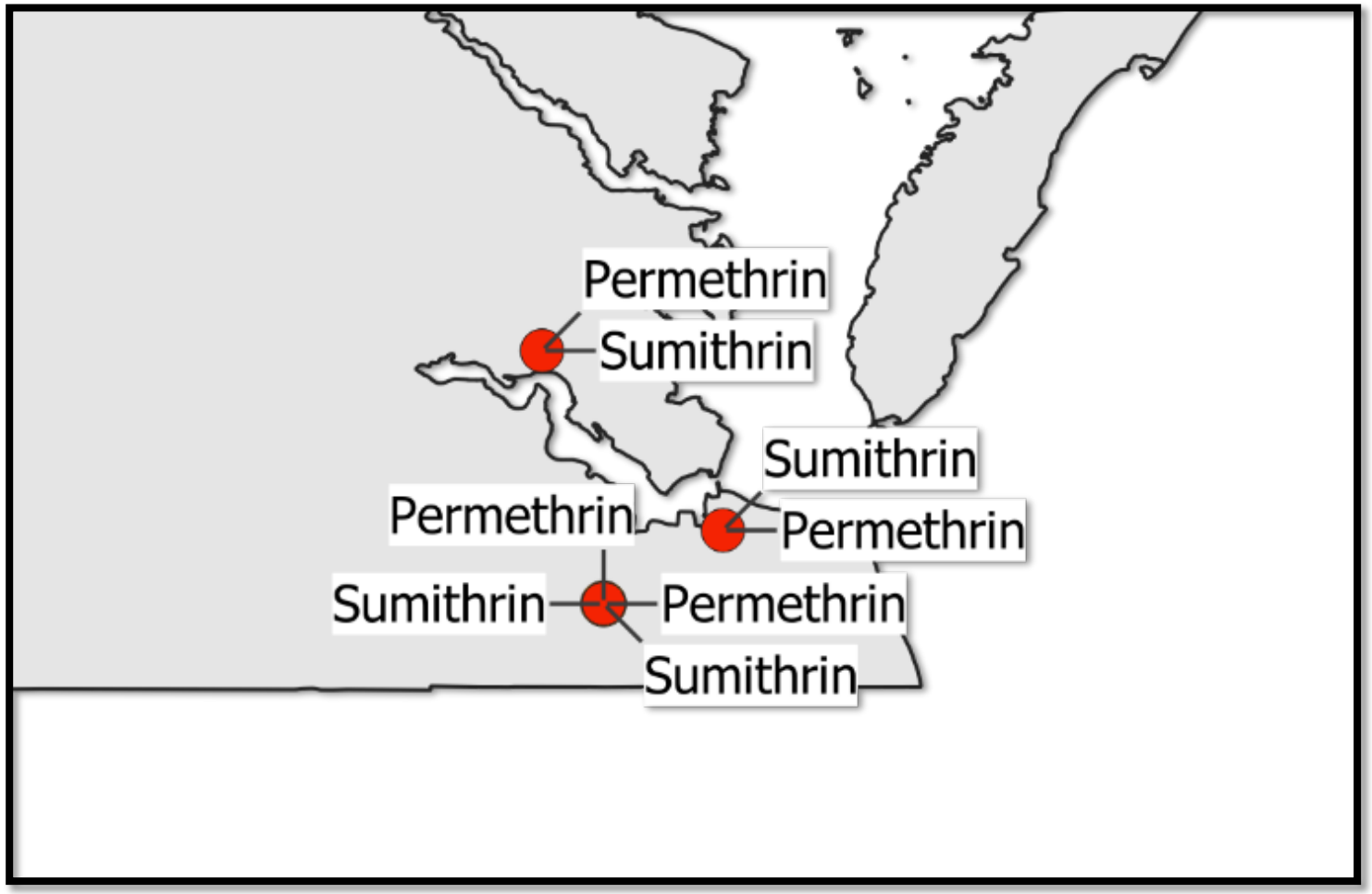
Interactive Map Available

https://www.neregionalvectorcenter.com/resistance

scan ME

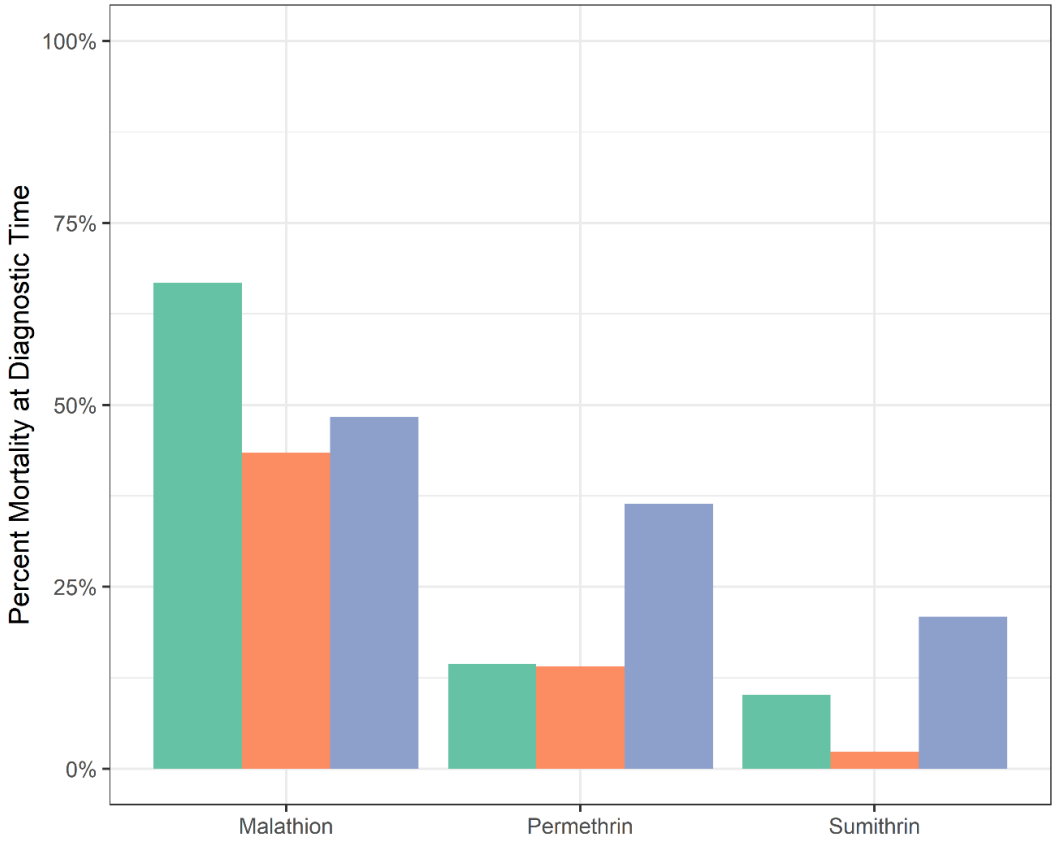


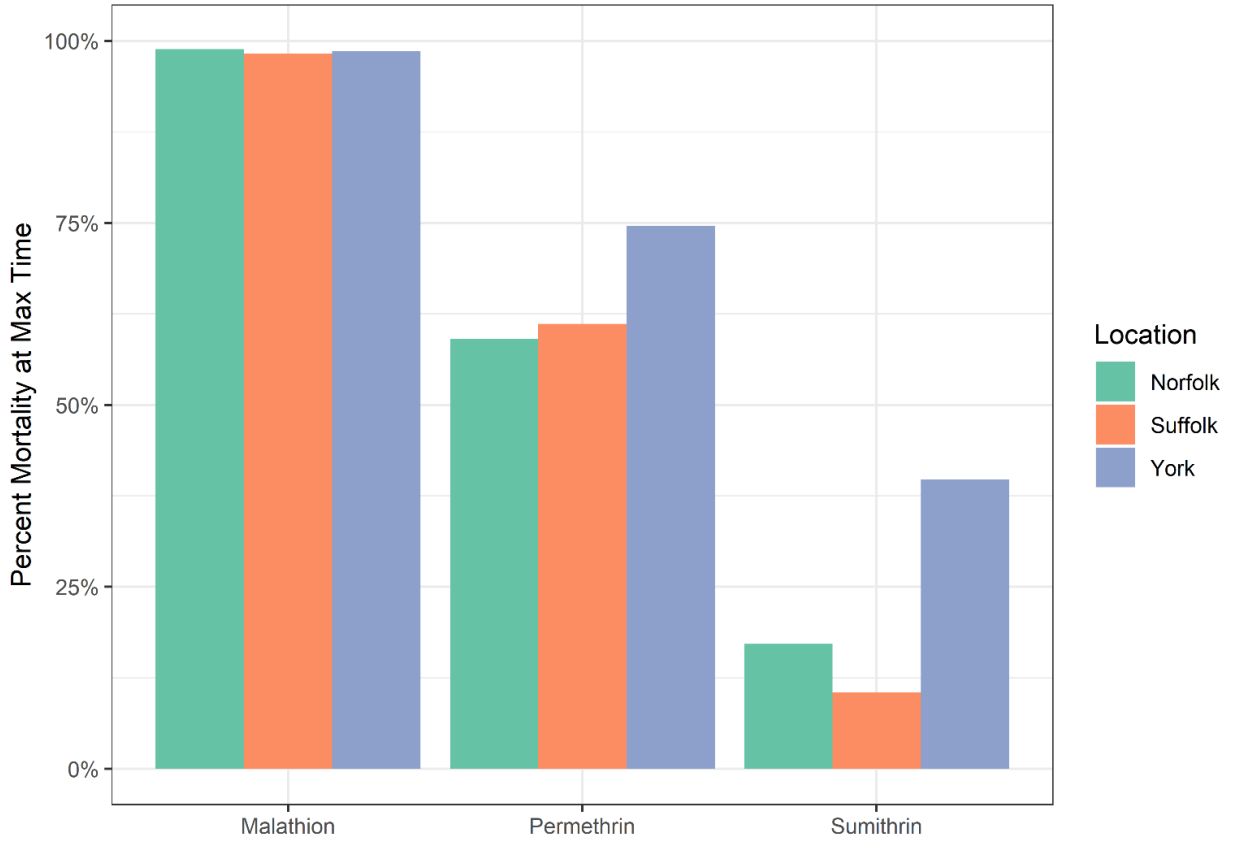
Resistance in Virginia



Low Mortality in *Culex pipiens* 2022

CDC bottle bioassay





Mechanism of Resistance

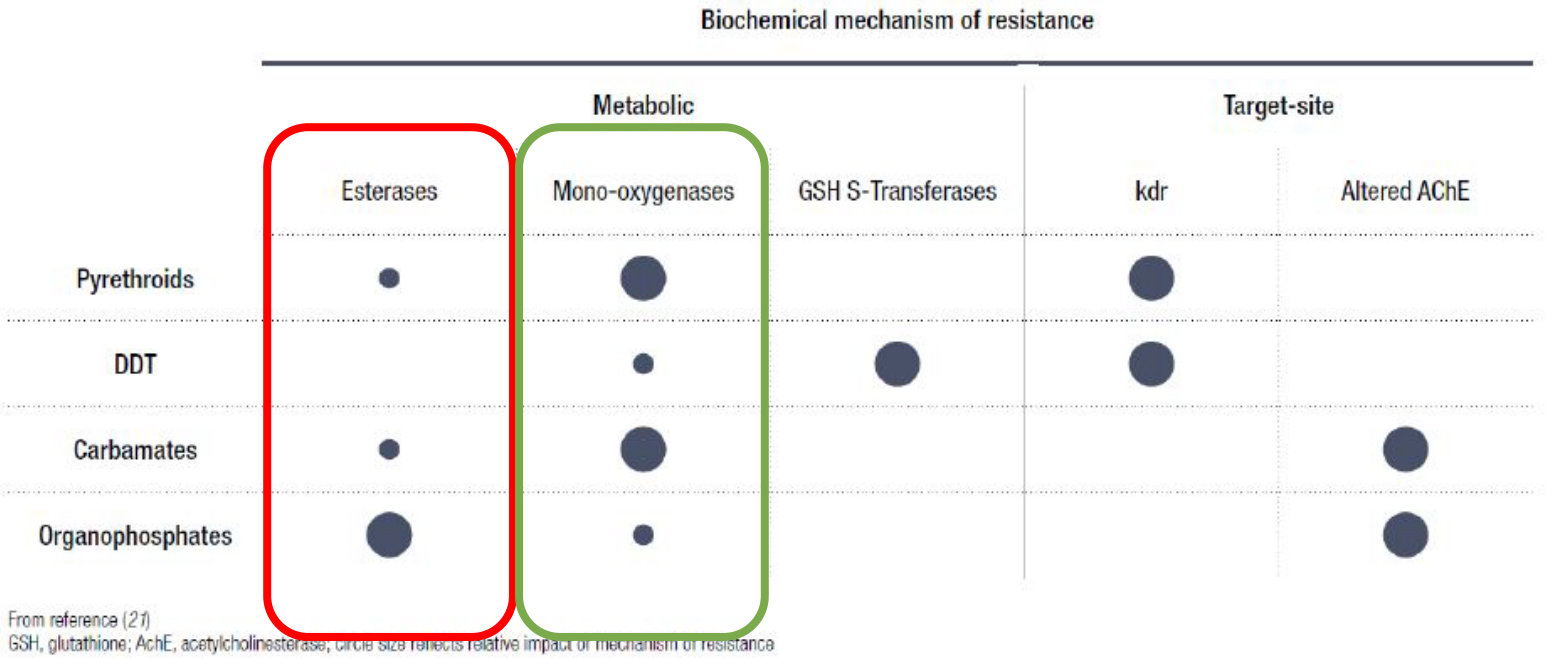
Resistance Ratios

Mechanism Testing *Culex pipiens* 2022

DEF: Esterases

PBO: Mono-oxygenases (P450's)

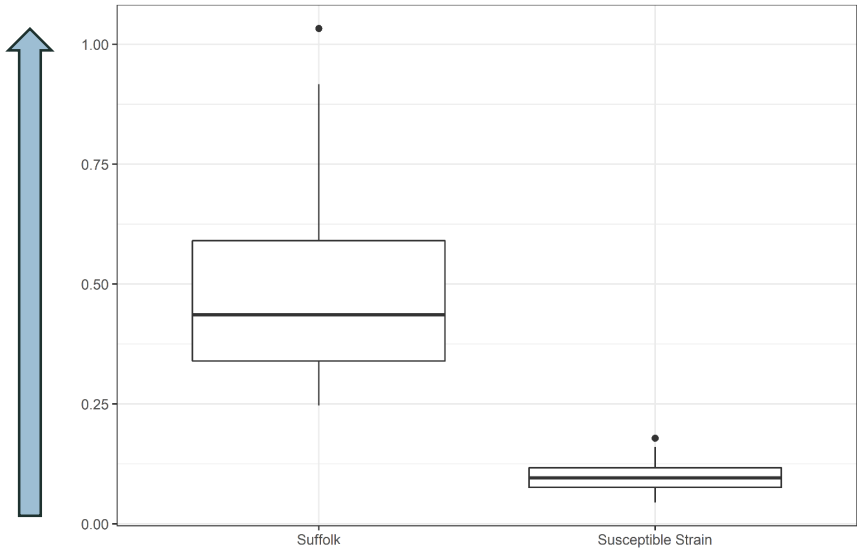
Cross resistance patterns of different classes of insecticide



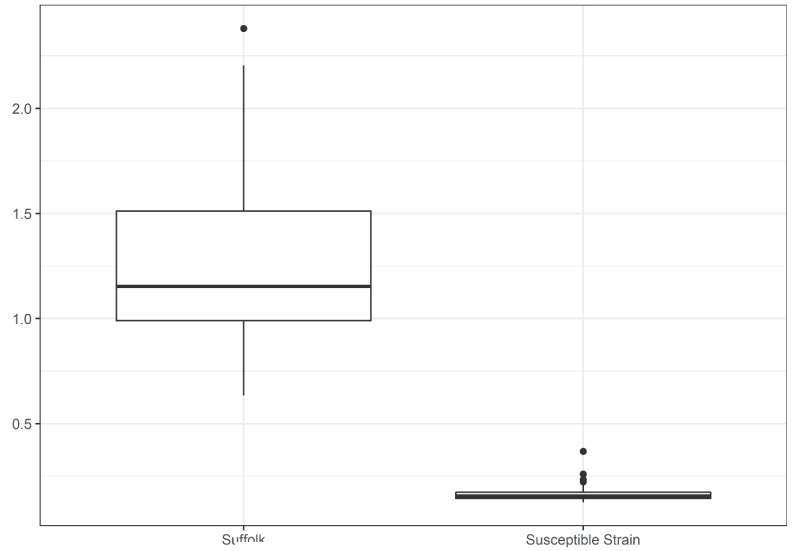
From reference (*21*)
GSH, glutathione; AchE, acetylcholinesterase; circle size reflects relative impact of mechanism of resistance

GPIRM Global Plan for Insecticide Resistance Management in malaria vectors. WHO, 2012

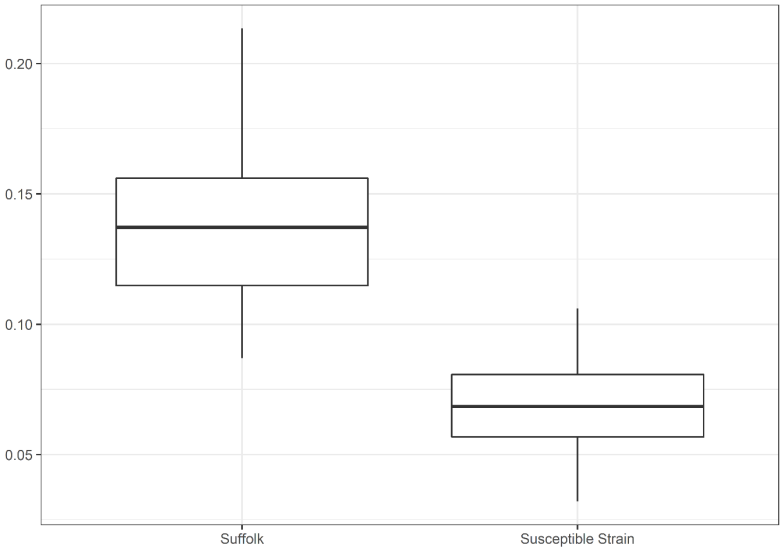
Elevated esterase and oxidase enzymes indicates less susceptible population



Alpha Esterase



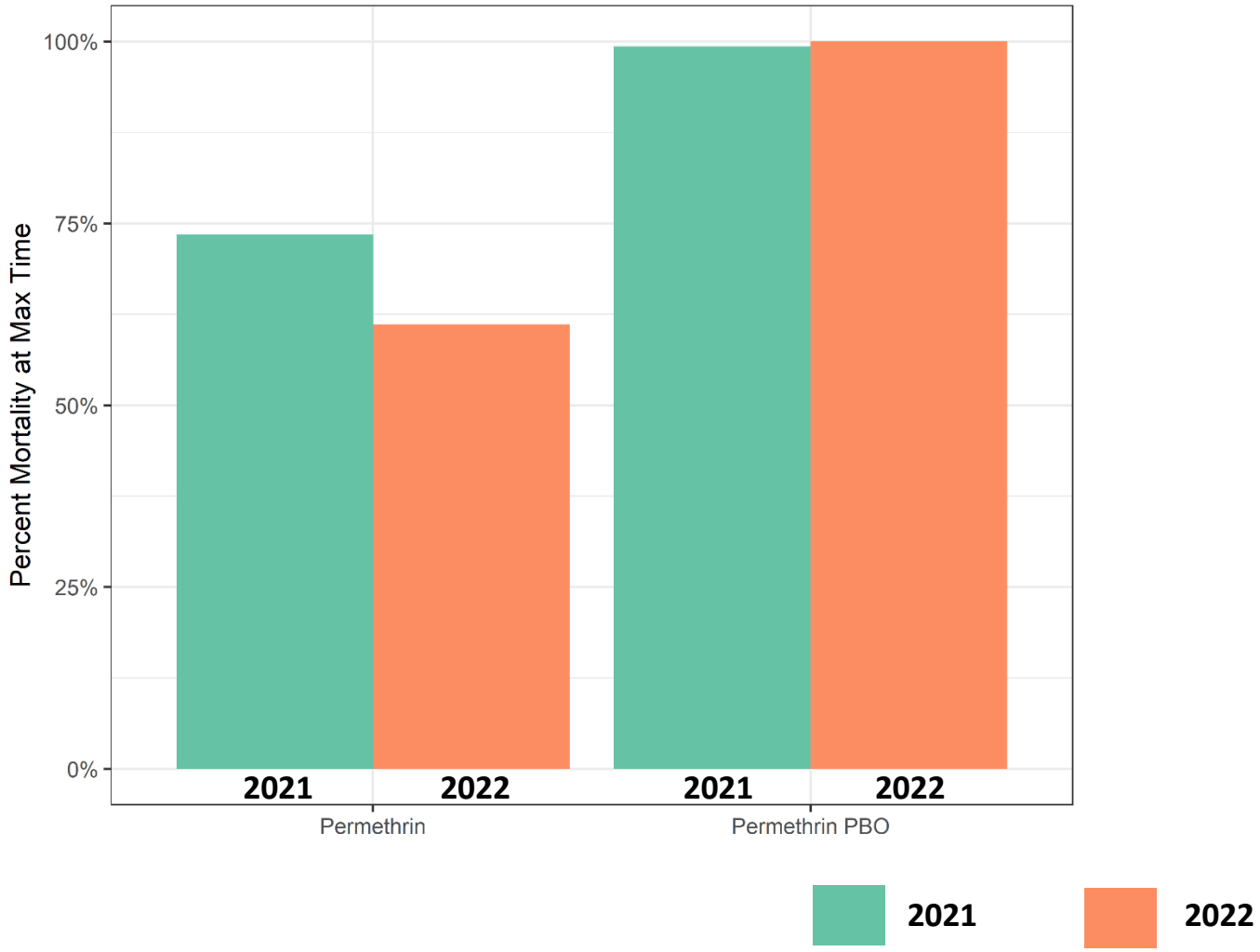
Beta Esterase



Oxidase

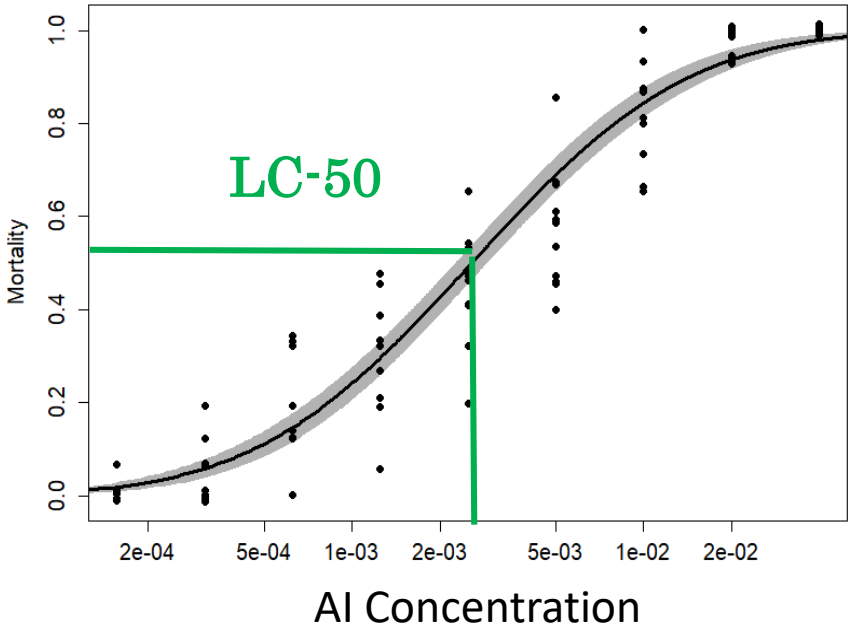
Reduced susceptibility to sumithrin in 2022

Mortality below 90% at max time is considered high level resistance



Resistance Ratios

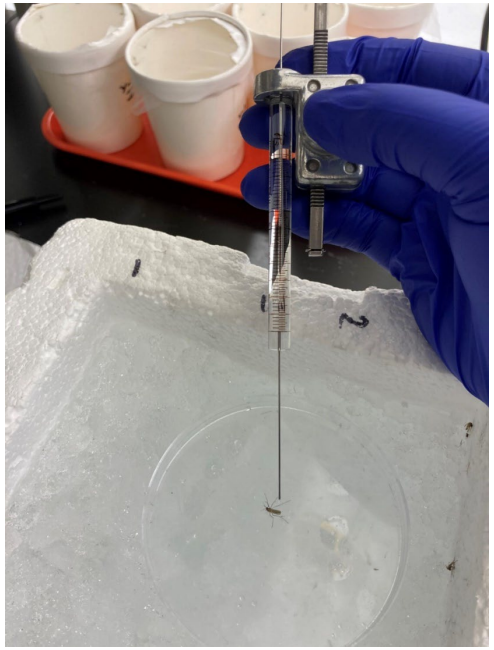
$$\frac{\text{LC50 Field Population}}{\text{LC50 Susceptible strain}} = \text{RR}$$



A RR of less than 5 is a susceptible population

Between 5 & 10 – Moderate resistance

Greater than 10 – Highly resistant



Lethal Concentrations (LC50)

AI	York	Suffolk	Norfolk	Susceptible
Permethrin	0.169	0.607	0.443	In progress
Sumithrin	0.275	0.766	0.634	0.00263
Malathion	6.809	3.355	5.087	In progress

Conclusions

- Elevated enzymes responsible for metabolic resistance in southern Virginia
- Low to moderate resistance to organophosphates
- Multi-year resistance data from set surveillance locations is helpful

Available on our interactive map

- Spotty, low to high level resistance to pyrethroids across the region in *Culex pipiens*
- Consistent low level resistance to methoprene in *Culex pipiens*
- No resistance to *Bti* or *Ls* detected in *Culex pipiens* or *Aedes albopictus*
- No resistance to pyrethroids detected in *Aedes albopictus* in 2022

Thanks!

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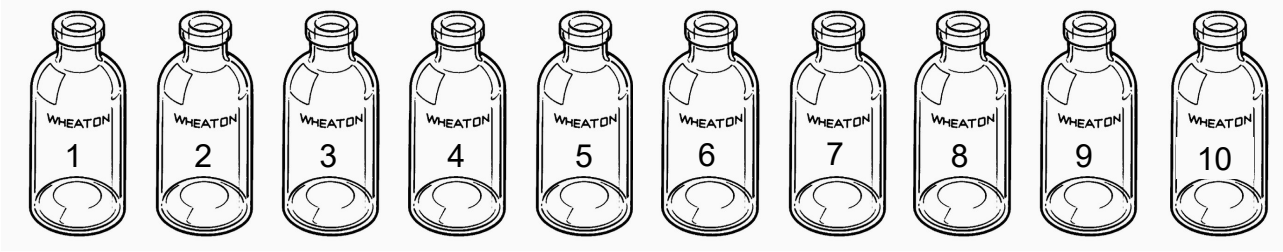
FAQs about CDC bottle bioassays

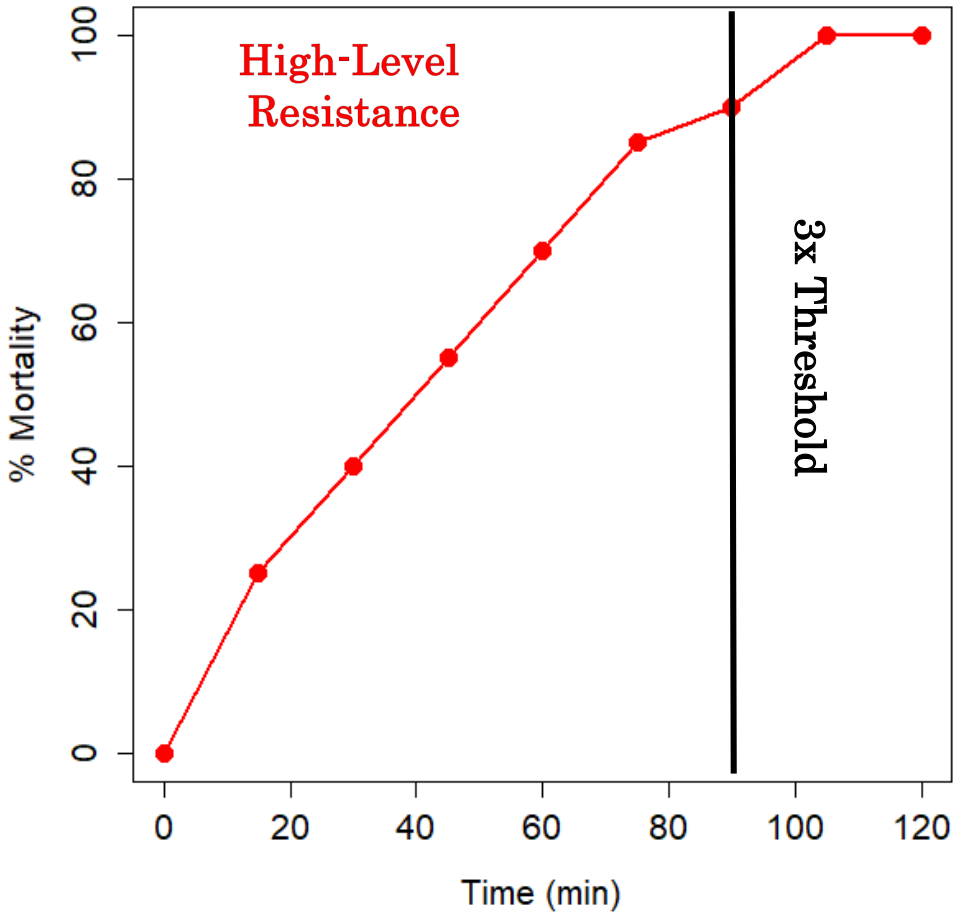
- Does etofenprox really dissolve in acetone? If not, it would lead to inaccurate results and false positives.
  - Yes. Etofenprox does dissolve readily in acetone. We do not detect false positives. Our bioassays are carefully conducted with field mosquitoes of uniform age and body size tested against a susceptible laboratory strain.
- How do you know that CDC bottle bioassays will represent resistance in field mosquitoes?
  - We carefully control factors that could influence our results. We only test mosquitoes collected in the field. We ensure that the assay is sensitive by testing our susceptible laboratory strain.
- Since light breaks down methoprene, how can you ensure the bioassays work?
  - We conduct our assays in the laboratory by treating water with methoprene and holding larvae in containers with the methoprene water under dark conditions.

CDC Bottle Bioassay Methods

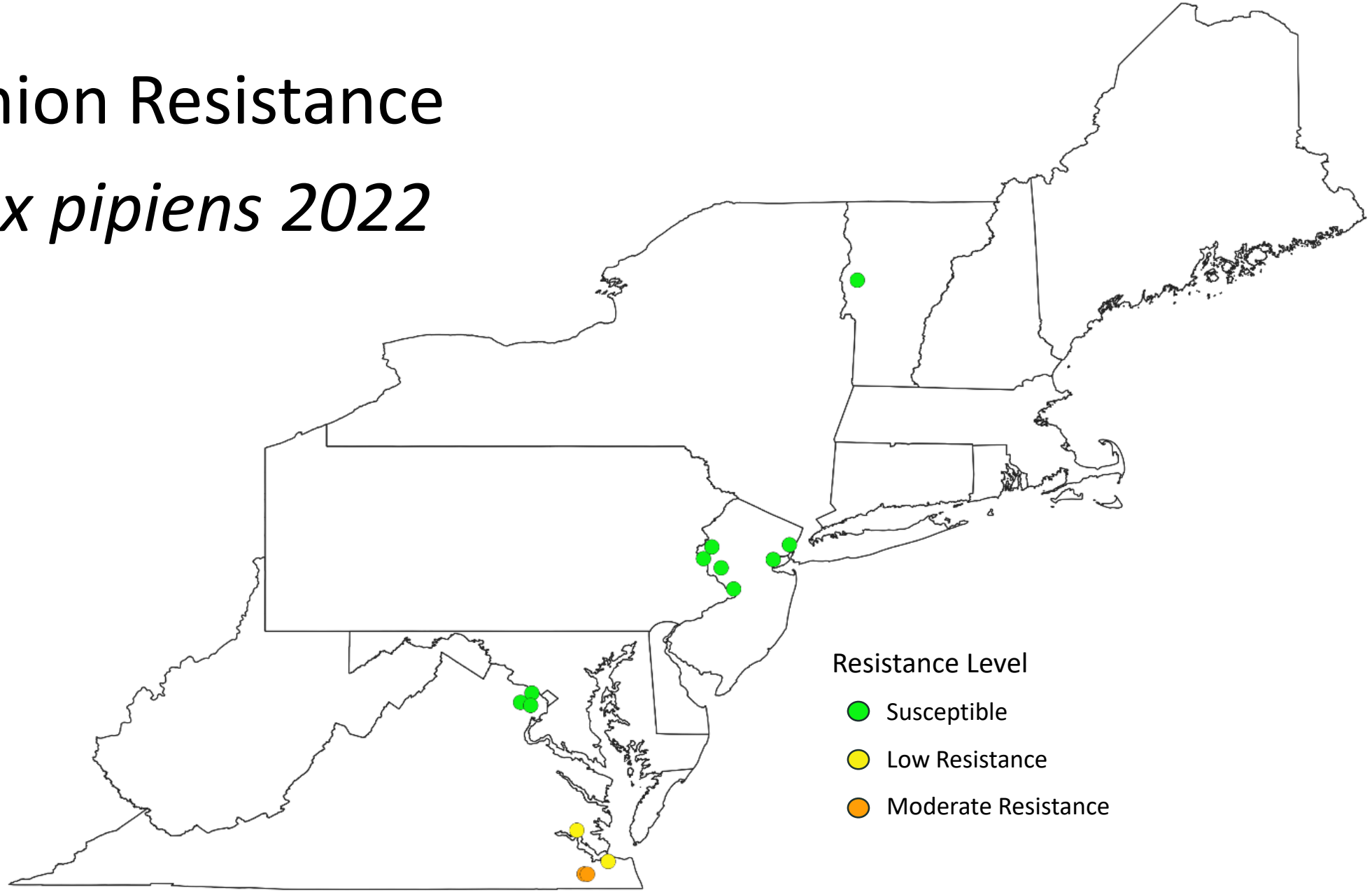
1. Control bottle is treated with the solvent (acetone) and is otherwise handled the same as the other bottles
2. 10 bottles are treated with 1ml of acetone containing a CDC-determined dose of active ingredient and allowed to dry while protected from light



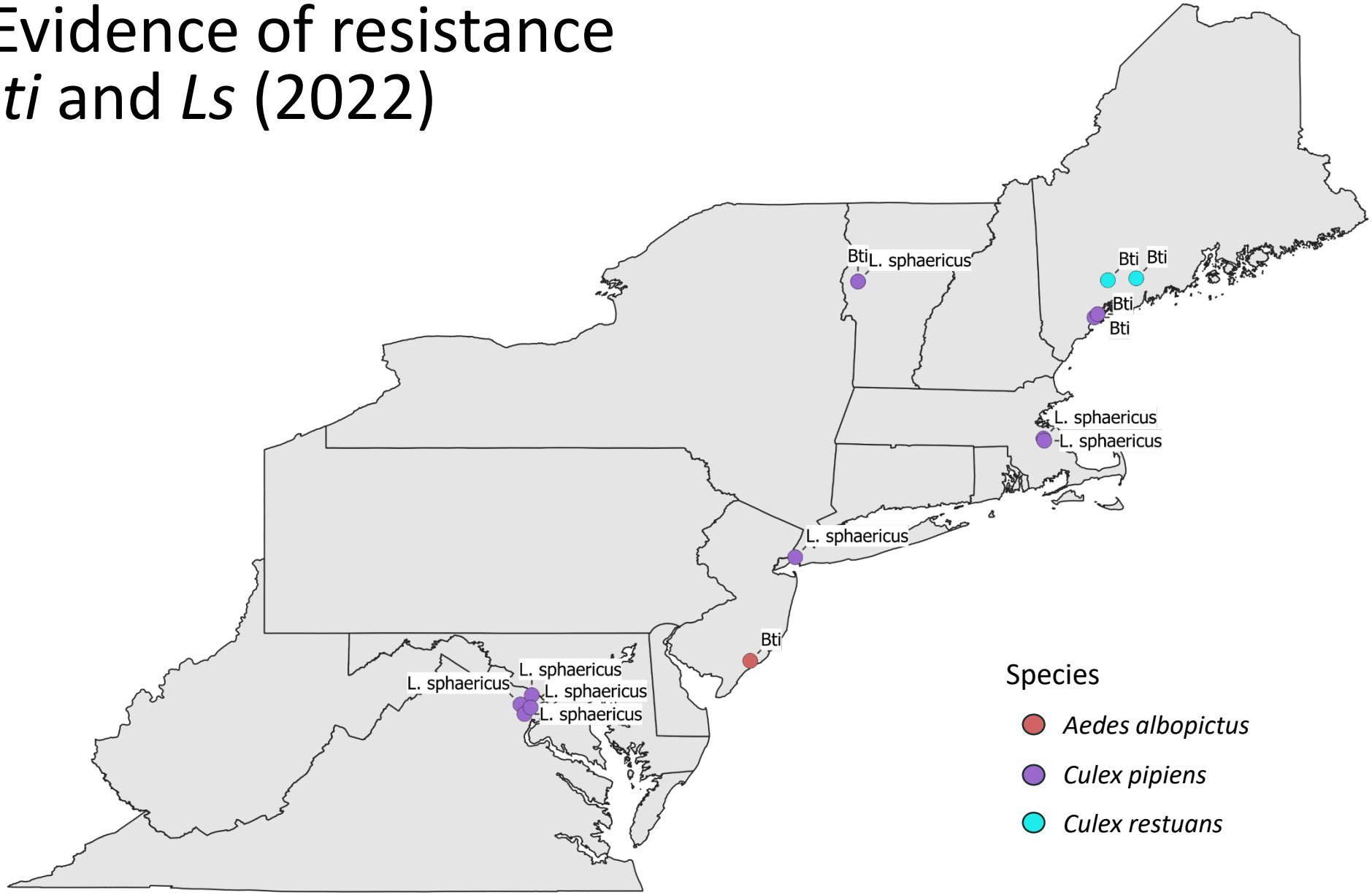
3. 25 3-5 day old female mosquitoes are introduced to each of the bottles
4. Mortality is recorded starting at the diagnostic time and may be monitored further until desired threshold mortality is achieved



Malathion Resistance in *Culex pipiens 2022*

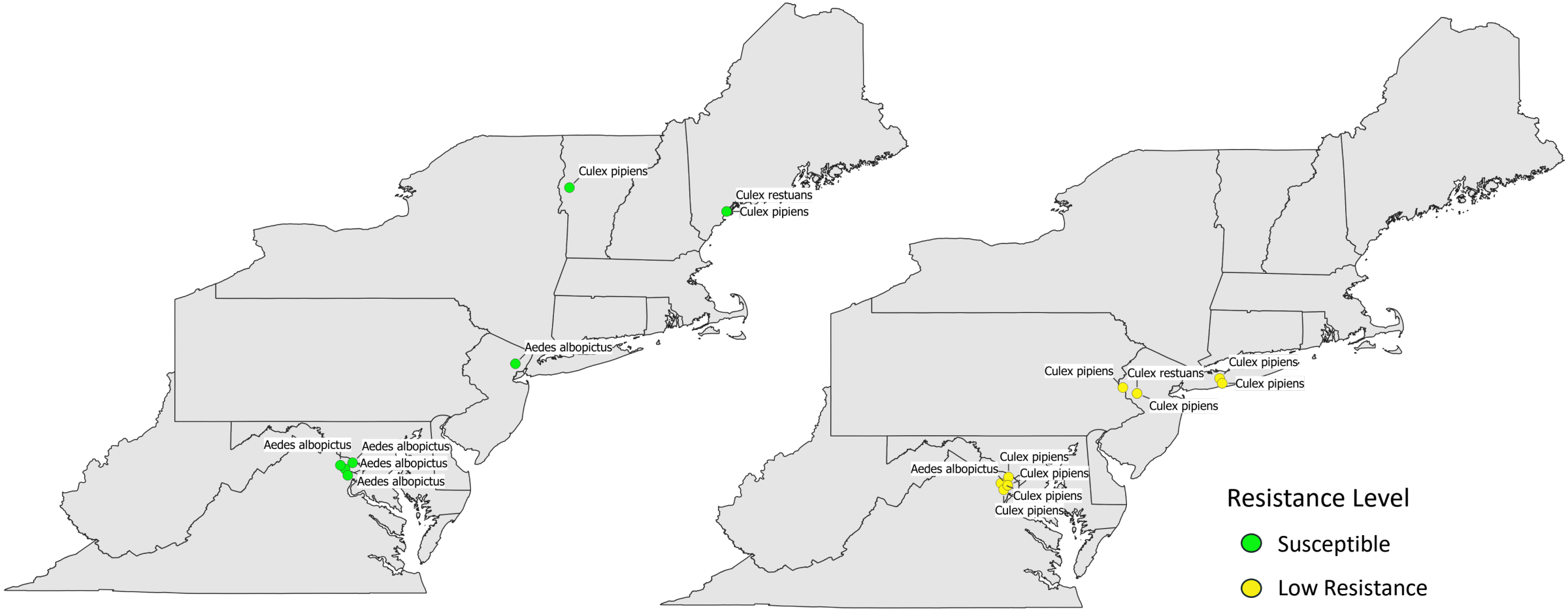


No Evidence of resistance to *Bti* and *Ls* (2022)



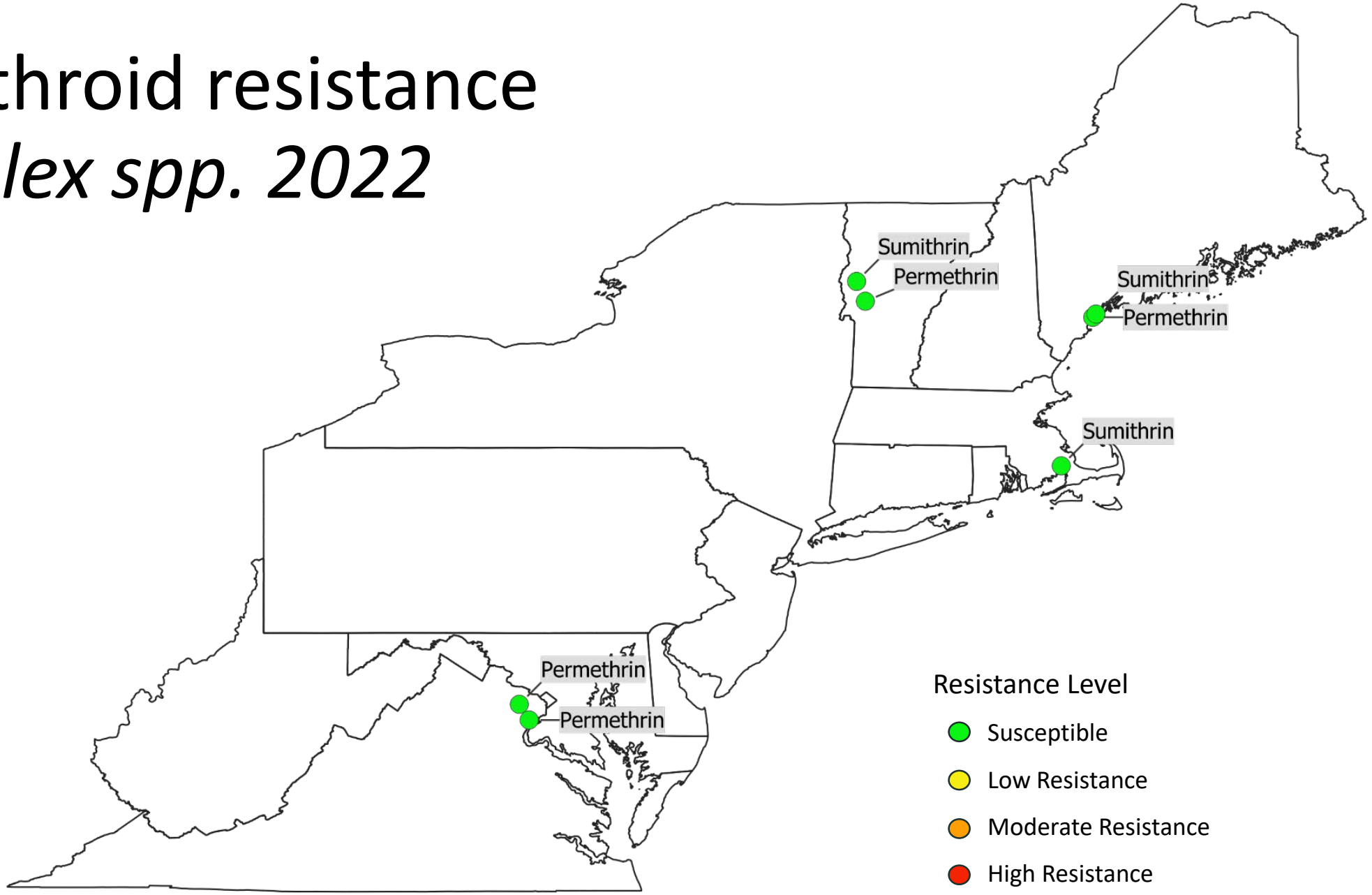
Low level Methoprene resistance in *Culex spp.* 2022

Routine larval bioassay

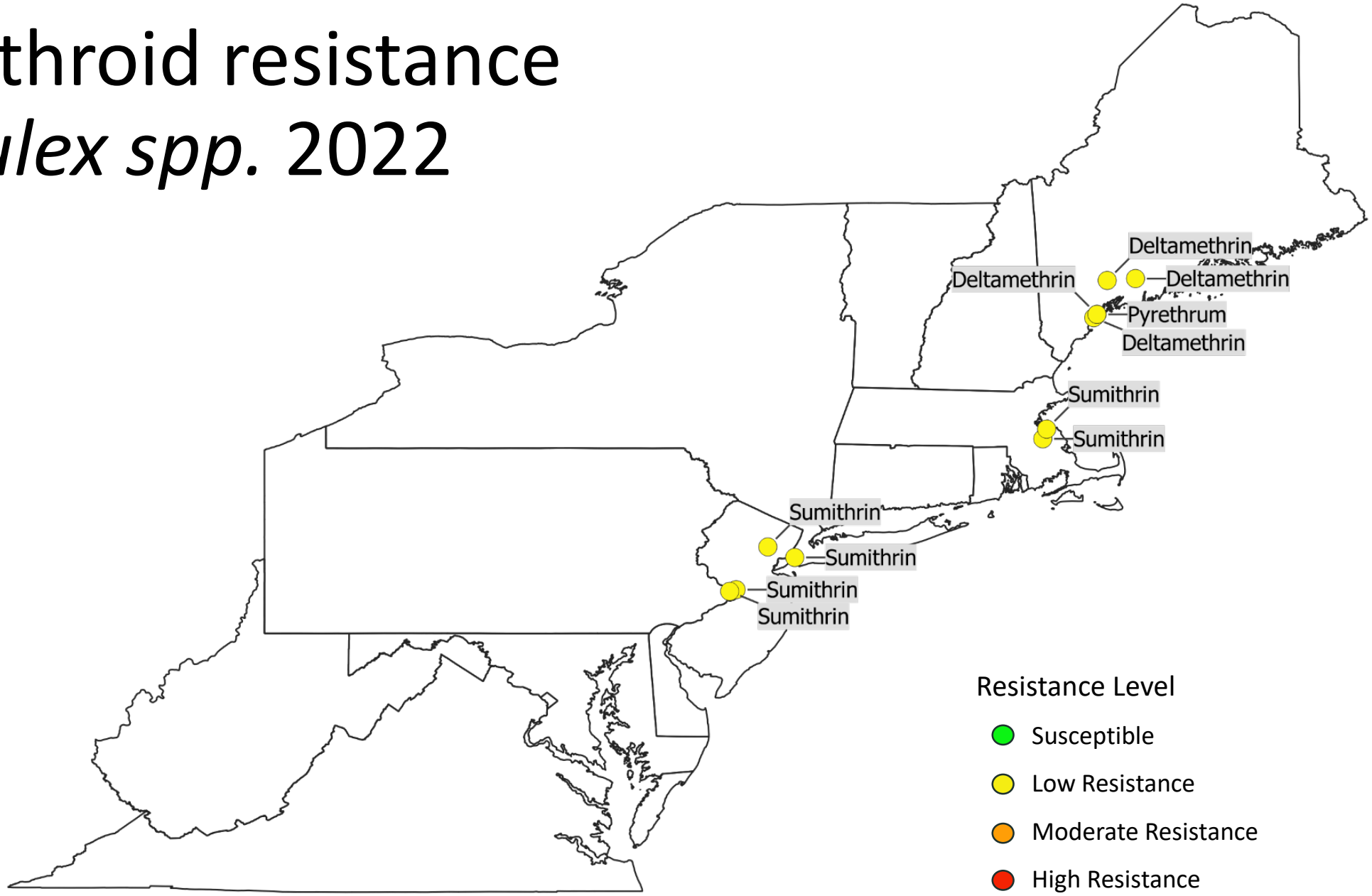


Etofenprox resistance in *Culex spp.* 2022

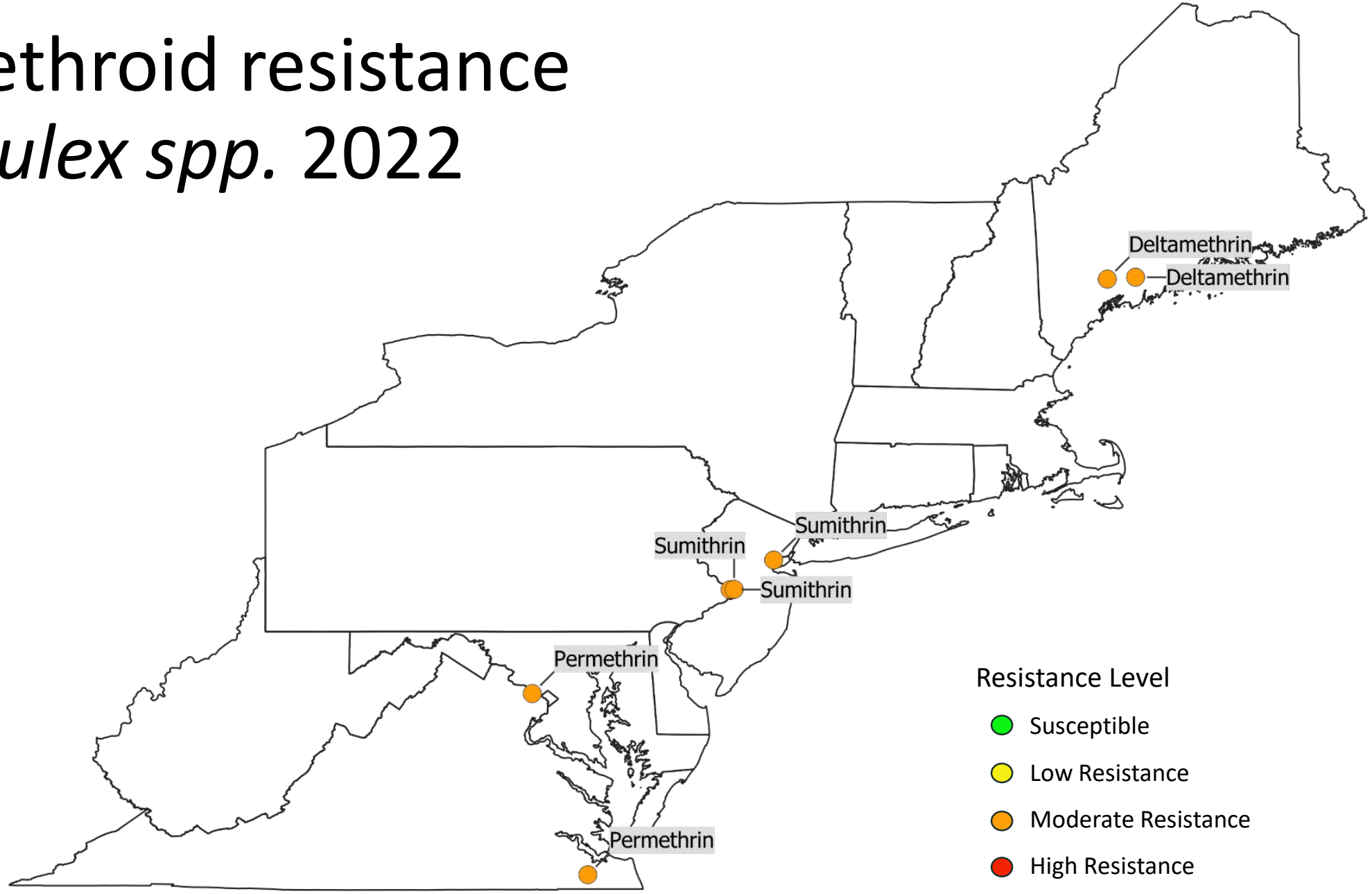
Pyrethroid resistance in *Culex spp. 2022*



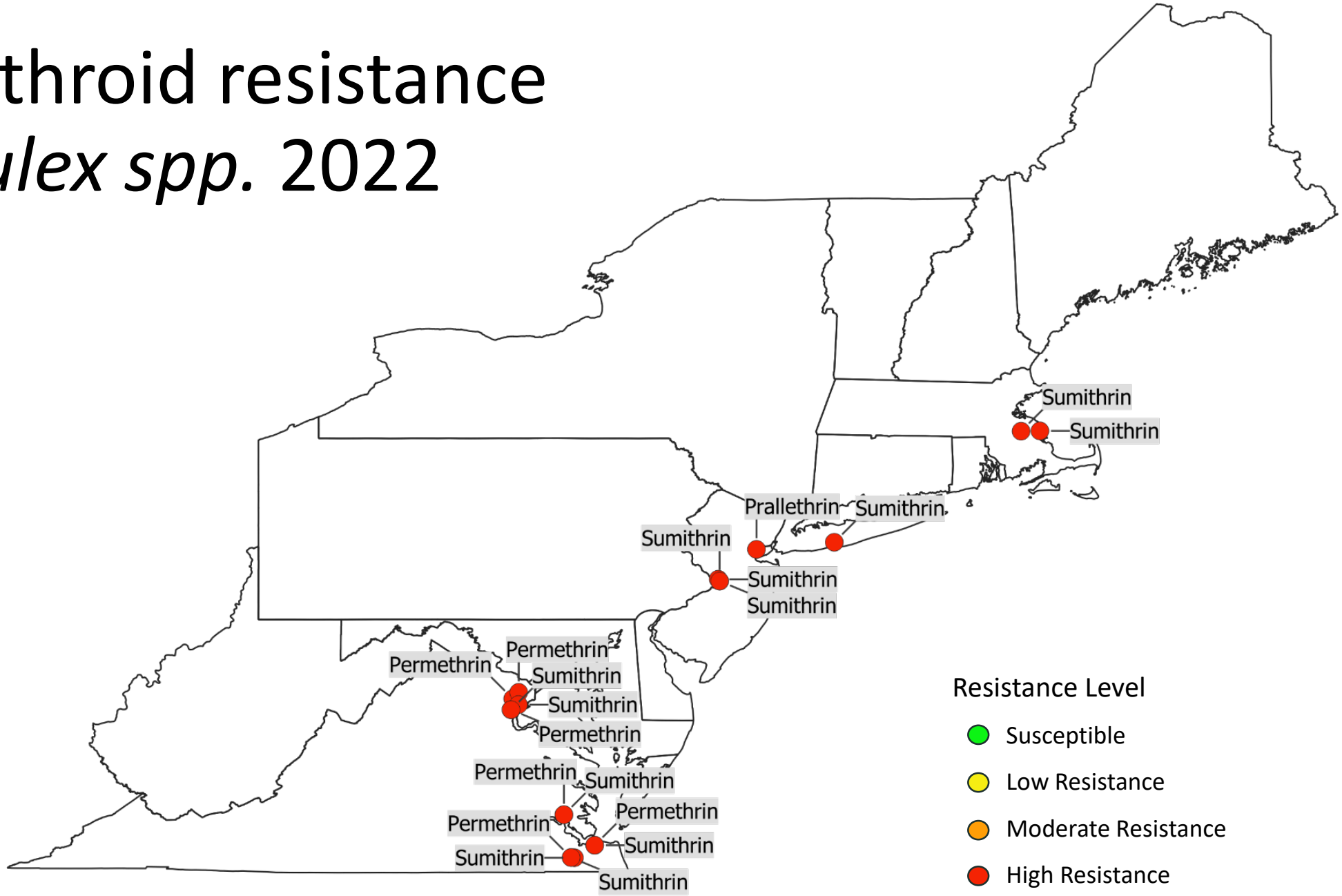
Pyrethroid resistance in *Culex spp.* 2022

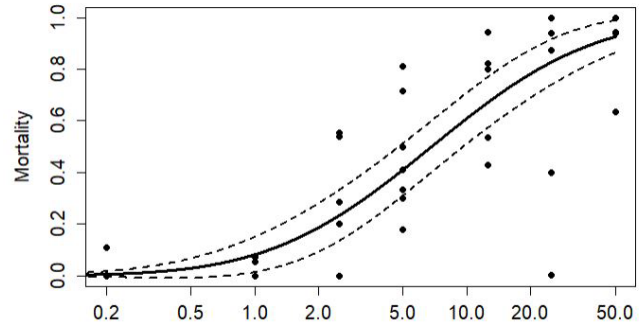
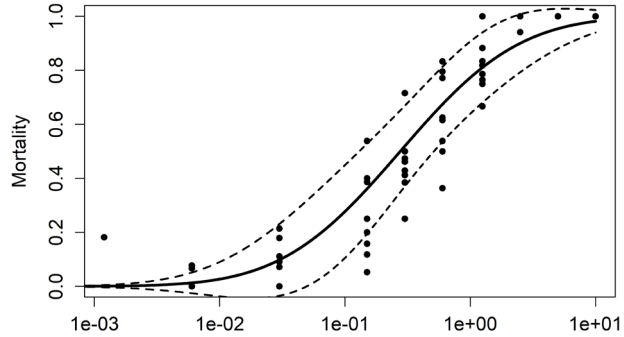
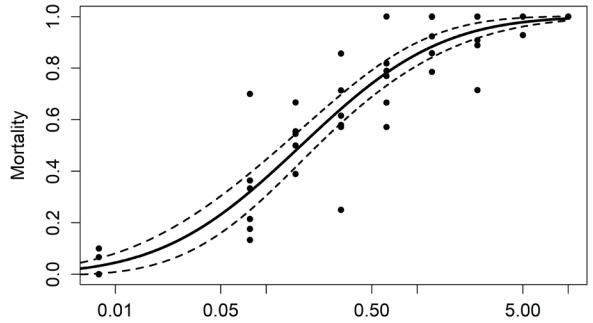
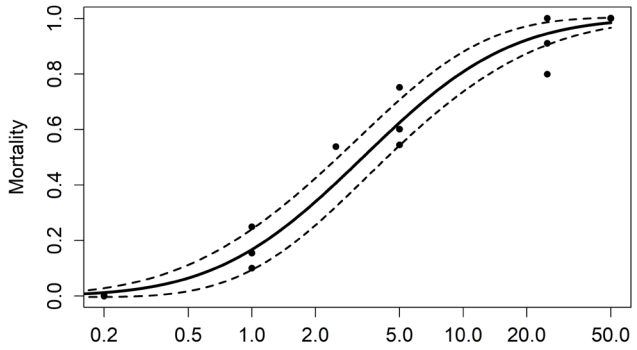
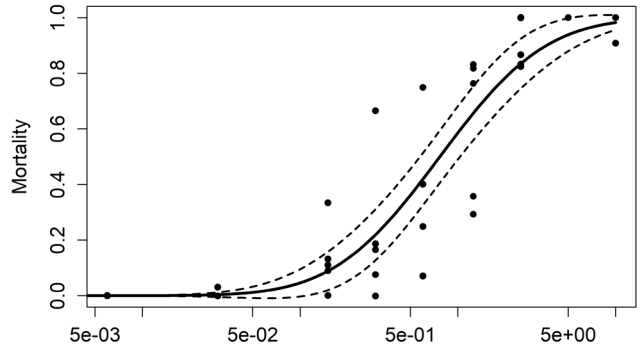
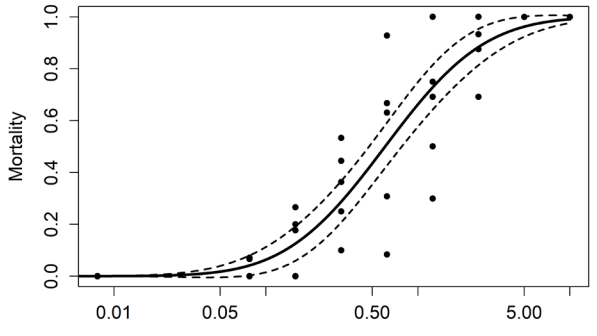


Pyrethroid resistance in *Culex spp.* 2022



Pyrethroid resistance in *Culex spp.* 2022



	Malathion	Sumithrin	Permethrin
York			
Suffolk			
Norfolk