



FAIRFAX COUNTY HEALTH DEPARTMENT

Getting an “egg” up on resistance testing in Fairfax County, VA—2022

VMCA Annual Conference

February 14 – 16, 2023

Andy Lima, Environmental Health Specialist III

Disease Carrying Insects Program



Project Goals

- Collect eggs of *Culex pipiens* and *Aedes albopictus*
- Improve upon pre-pandemic efforts (2019)
- Ship immatures to NEVBD for resistance testing
- Determine baseline level insecticide resistance in Fairfax County mosquitoes



Presentation Goals

- Describe egg collection efforts of 2022
- Share successes and failures of:
 - Experimental design
 - Collections
 - ID of immatures
 - Sample shipment
- Summarize resistance testing results



Testing Services offered by NEVBD



Photo: CDC bottle bioassay manual



Adult resistance testing

CDC bottle bioassay

Active ingredients

- Chlorpyrifos - Deltamethrin - Etofenprox - Fenthion - Malathion - Naled - Permethrin - Prallethrin - Pyrethrum - Sumethrin - Bifenthrin

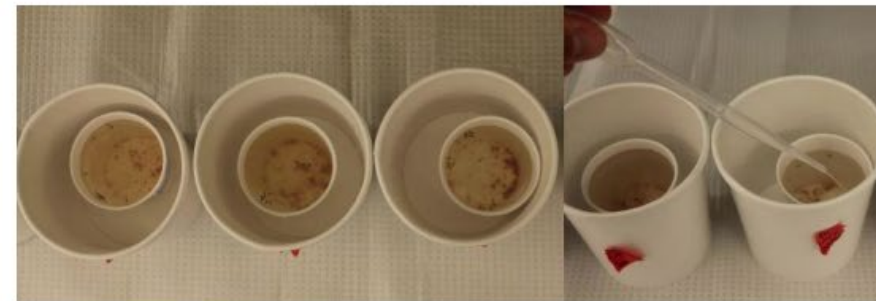
Inhibitors

- PBO - DEF - DM

Larval resistance testing

Active ingredients

- Methoprene - Bti - L sphaericus



NEVBD Pesticide Resistance Monitoring Overview 2022.PDF (Cornell E-Commons)

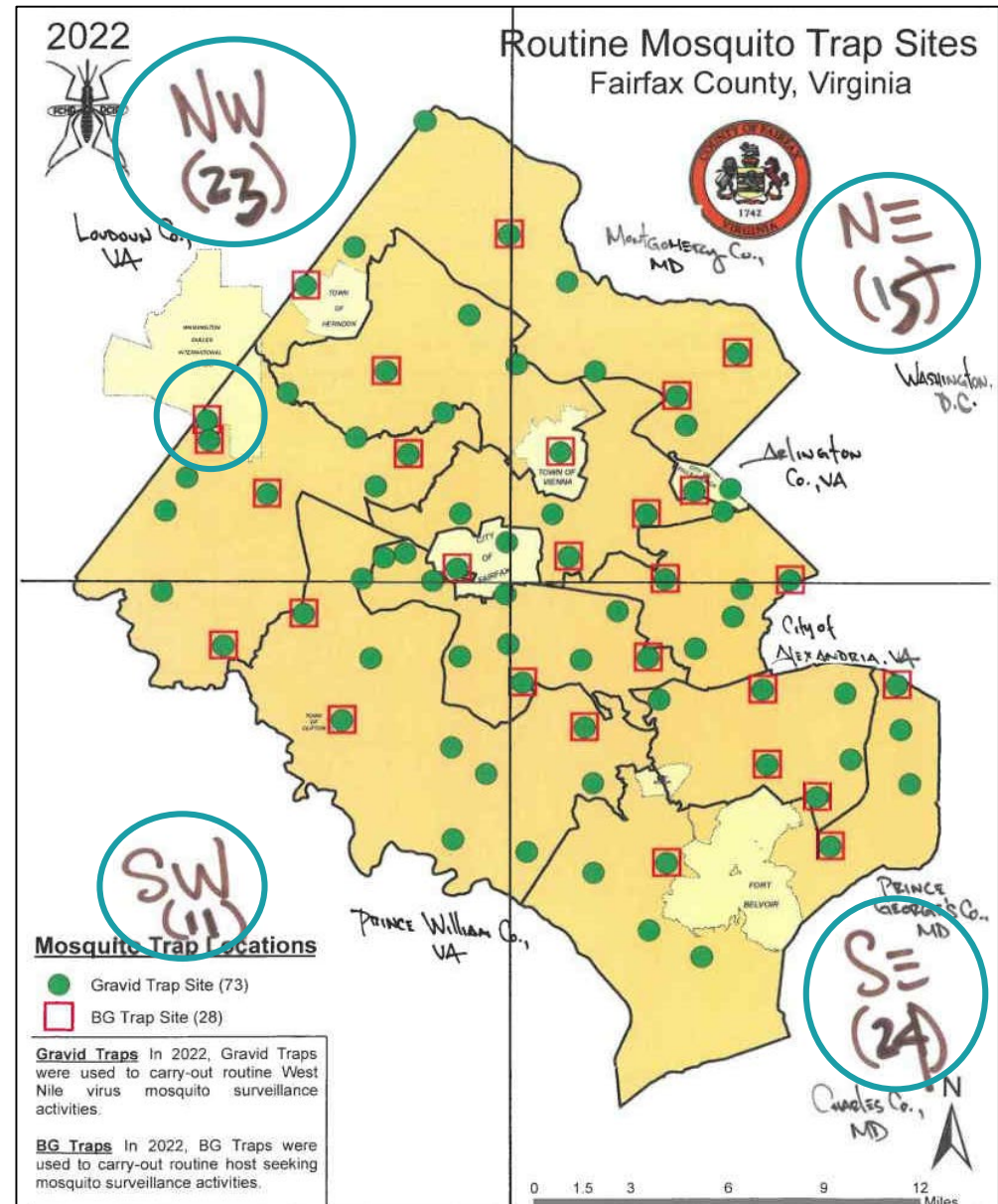


FAIRFAX COUNTY HEALTH DEPARTMENT



Mosquito Surveillance Network

- 73 routine gravid trap sites
- 28 routine gravid trap sites
- Quadrants created
- 1 additional site selected
 - **Police Training Facility**



Site Representation for Egg Collections

Quadrant	# Routine Trap Sites	# <i>Cx. pipiens</i> egg Sites	<i>Cx. pipiens</i> , prop. sites represented	# <i>Ae. albopictus</i> egg sites	<i>Ae. albopictus</i> , prop. sites represented
Northwest	23	12	52%	7	30%
Northeast	15	11	73%	5	33%
Southwest	11	6	55%	6	55%
Southeast	24	22	92%	3	13%

~70% of sites represented in *Cx. pipiens* egg collections.

~30% of sites represented in *Ae. albopictus* egg collections.



How many mosquitoes do we need to collect?

CDC Bottle Bioassay

- Etofenprox, Permethrin
 - Bonus Malathion
- 500 eggs or larvae / AI
 - 250 adult females / bioassay
- Need ~1,000 eggs or larvae per species per quadrant

Larval Bioassay

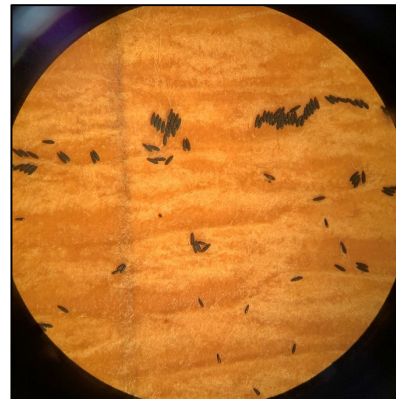
- Bti, Ls, Methoprene
- 300 eggs or larvae / AI
- Need ~900 eggs or larvae per species per quadrant



Target: **~10,000** of each species

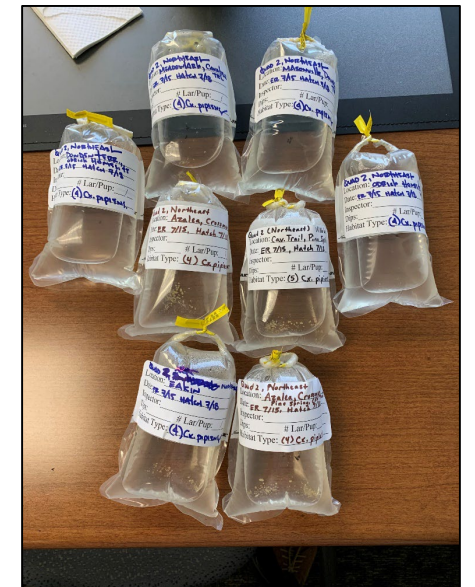
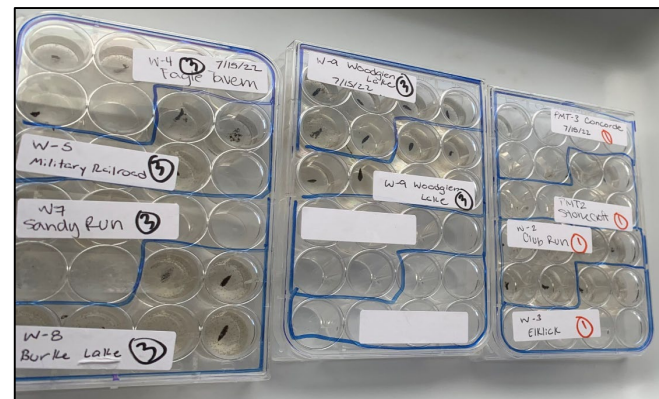
Ae. albopictus

- ~9,200 eggs from 22 locations
 - 8/12/22 – 8/22/22
 - 8/26/22 – 9/1/22



Cx. pipiens

- ~22,000 larvae from 51 locations
 - 7/15/22
 - 7/20/22

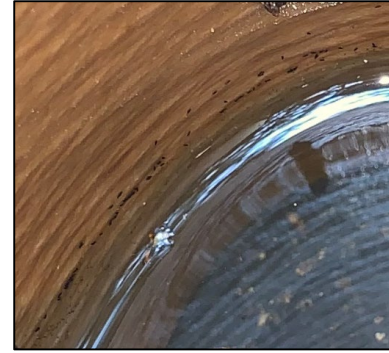


Egg collection methodology, *Cx. pipiens* vs. *Ae. albopictus*



Cx. pipiens

- 1 bus bin per site
- 1 gallon Gravid water
 - Fresh grass, straw/hay, brewer's yeast
- Overnight deployment
- Collect egg rafts
- Hatch in lab and ship to Cornell



Ae. albopictus

- 5-10 drinking cups per site
- 2 papers per cup
- 8 oz. dechlorinated water with pinch of koi food
- Deployed 5-7 days
- Collect germination papers
- Ship to Cornell for hatch





Culex pipiens egg collections: Tips for success

- Collect early in the week

AUGUST 2022 A healthier community begins with you

I CAN'T FIGURE OUT WHY THERE ARE SO MANY SKEETERS THIS YEAR...

SMACK!

SUN.	MON.	TUES.	WED.	THURS.	FRI.	SAT.
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

WATER SLIDE

Always read and follow label instructions when using repellents.

Play golf? Ticks are hiding in the rough.

West Nile virus is present in Fairfax County every year.

Corrugated drain pipes used as downspout extensions, even if underground, are important breeding sites for mosquitoes. Remove and replace with something that doesn't hold water when possible. If above ground, empty them once a week. Otherwise, apply a larvicide to the inside of the pipe to prevent mosquitoes from breeding. Reapply the larvicide according to label instructions. Secure the larvicide inside the pipe to prevent it from being washed away.

Deer are the main system for ticks.

Fairfax County Health Department • Visit our Web page at www.fairfaxcounty.gov/health/fighthebite • fighthebite@fairfaxcounty.gov





Cx. pipiens egg collections: Tips for success

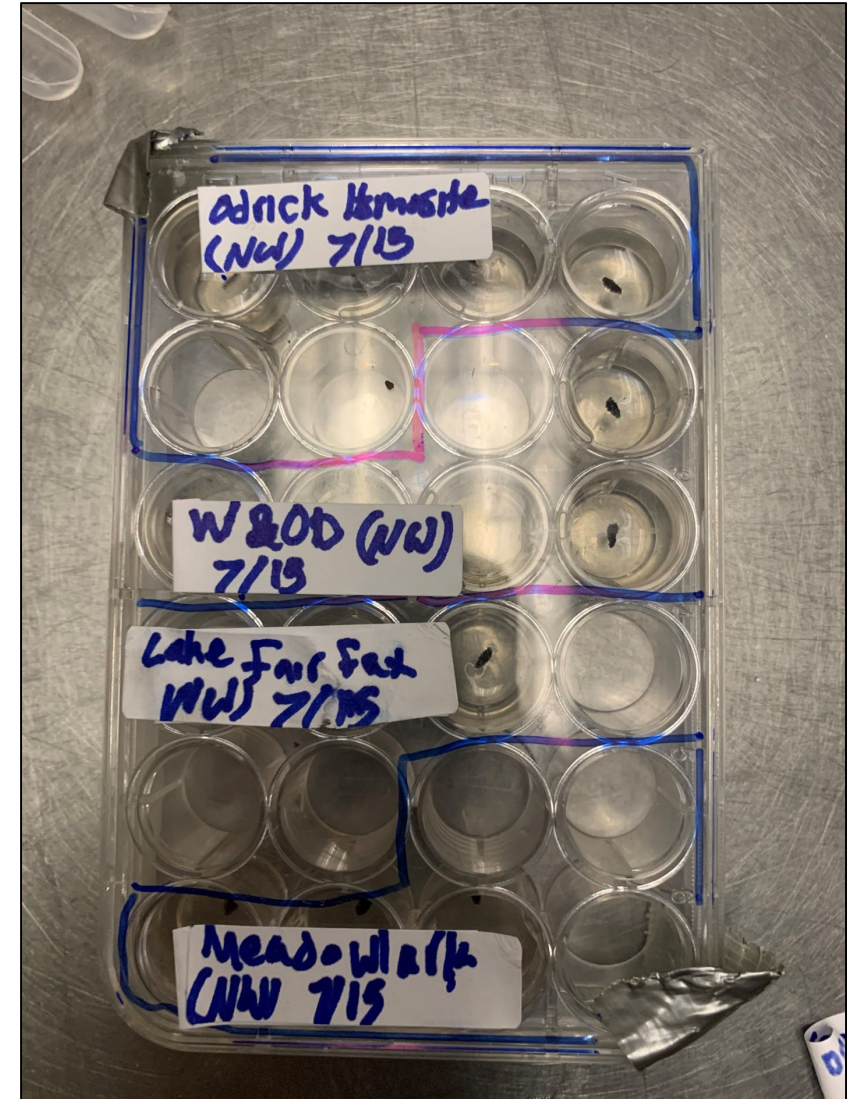
- Collect early in the week
- **Create pickup routes**





Cx. pipiens egg collections: Tips for success

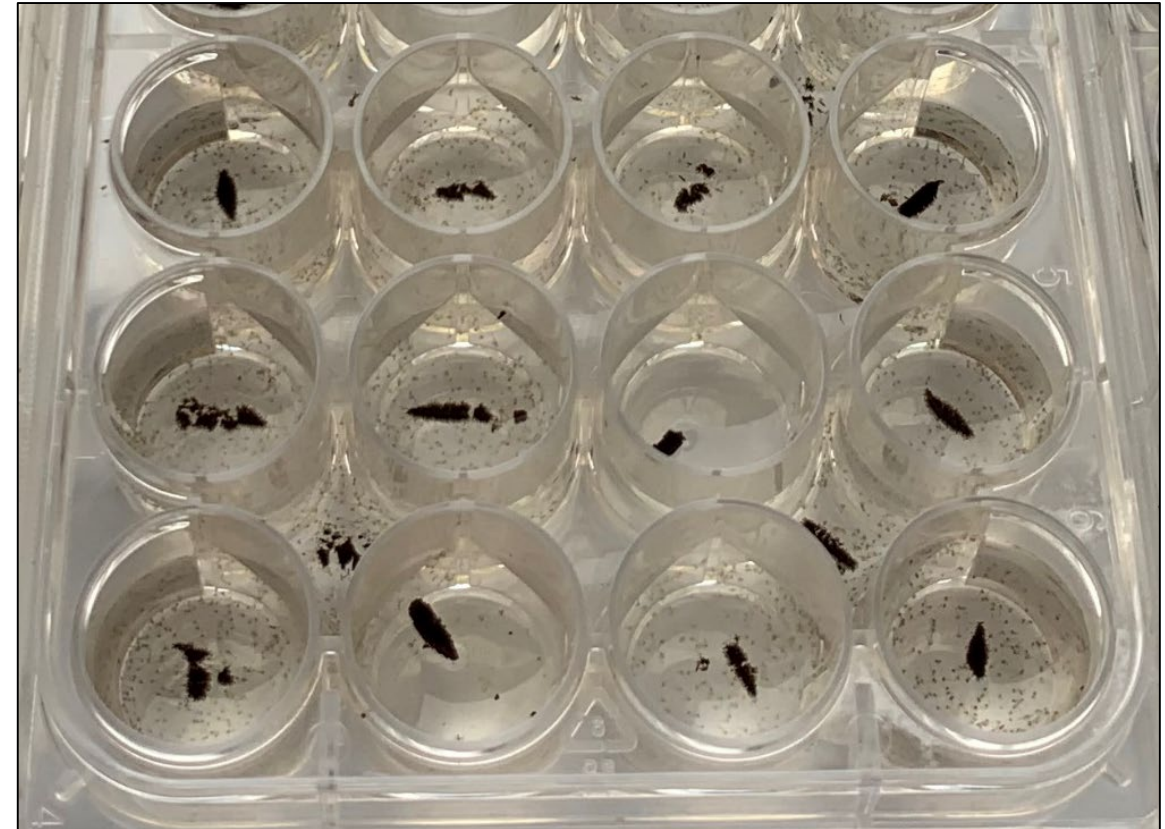
- Collect early in the week
- Create pickup routes
- **Label and tape well plates closed in field**





Cx. pipiens egg collections: Tips for success

- Collect early in the week
- Create pickup routes
- Label and tape well plates closed in field
- **One egg raft per well plate**



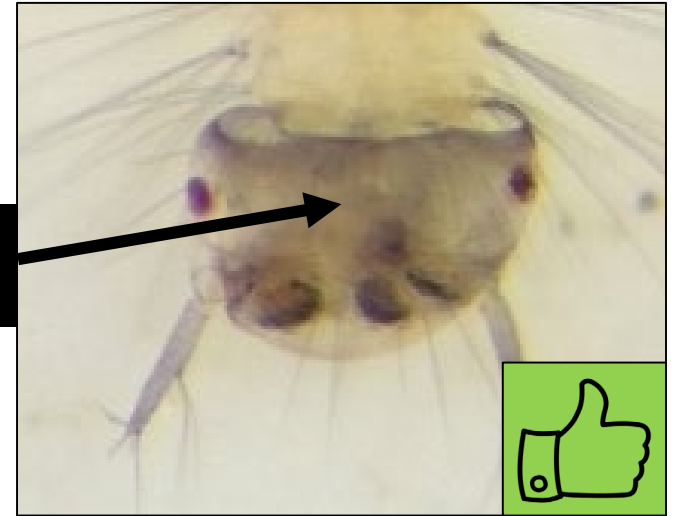


Cx. pipiens egg collections: Tips for success

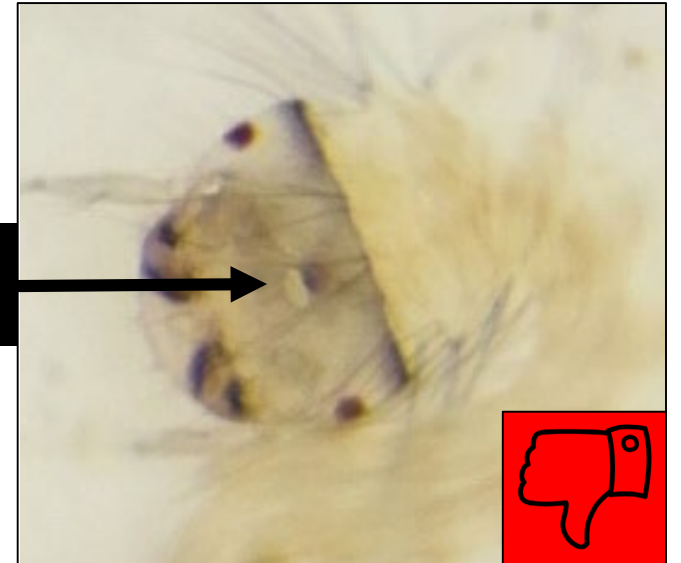
- Collect early in the week
- Create pickup routes
- Label and tape well plates closed in field
- One egg raft per well plate
- **Look for the window anterior to egg-breaker**

**ask Karen Akaratovic about exceptions.*

Window absent:
*Cx. pipiens**



Window present:
Cx. restuans





Cx. pipiens egg collections: Tips for success

- Collect early in the week
- Create pickup routes
- Label and tape well plates closed in field
- One egg raft per well plate
- Look for the window anterior to egg-breaker
- **Refrigerate for the weekend if necessary**
 - Give tiny pinch of food
 - 38-42 degrees, no more than 3 days





Culex pipiens egg collections: Challenges / Failures

- Leaving gravid water out for >1 day
- Multiple egg rafts in whirl-paks or wells
- Setting the fridge temp too low
- Poor labelling
- Early record-keeping
- Misunderstanding of shipping processes





Ae. albopictus egg set/pickup: Tips for success

- Prepare all supplies before departure





Ae. albopictus egg set/pickup: Tips for success

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- **Draw dark pencil lines/grids on egg papers**





Ae. albopictus egg set/pickup: Tips for success

- Prepare all supplies before departure
- Draw dark pencil lines/grids on egg papers
- **Create routes for set/pickup**
 - Document ovicup placement





Ae. albopictus egg set/pickup: Tips for success

- Prepare all supplies before departure
- Draw dark pencil lines/grids on egg papers
- Create routes for set/pickup
- **Bring extra water**





Ae. albopictus egg set/pickup: Tips for success

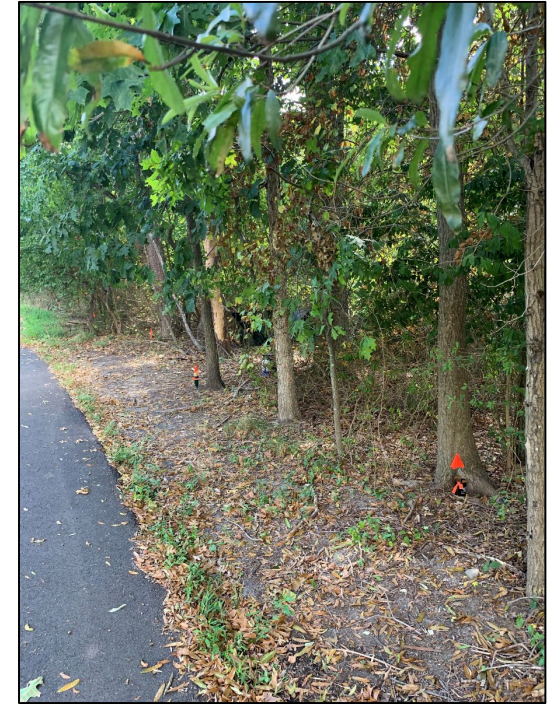
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- Bring extra water
- **Apply repellent at office**





Ae. albopictus egg set/pickup: Tips for success

- Prepare all supplies before departure
- Draw dark pencil lines/grids on egg papers
- Create routes for set/pickup
- Bring extra water
- Apply repellent at office
- **Select protected sites**



Ae. albopictus ovitrap anchored with construction flag

- Paperclips at edge or on top
- Gridlines on paper
- Loop of flagging tape
 - Flag slides through
- Stayed upright
- Easily visible





Ae. albopictus egg set/pickup: Tips for success

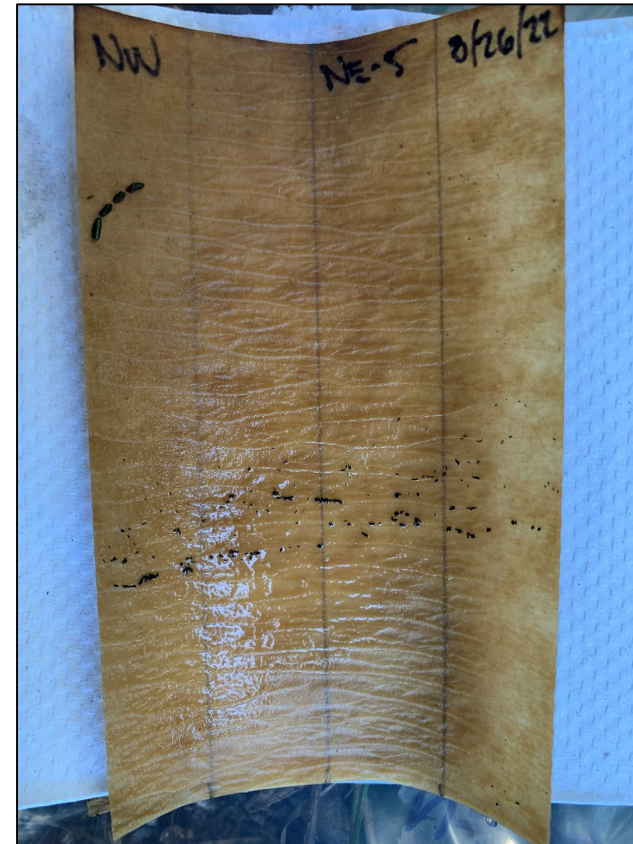
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- Select protected sites
- **Deploy 5-7 days and watch the weather**





Ae. albopictus egg set/pickup: Tips for success

- Prepare all supplies before departure
- Draw dark pencil lines/grids on egg papers
- Create routes for set/pickup
- Bring extra water
- Apply repellent at office
- Select protected sites
- Deploy 5-7 days and watch the weather
- **Remove excess moisture at pickup**



Ae. albopictus egg paper pickup





Ae. albopictus egg ID in the lab: Tips for success

- Process papers ASAP
- Monitor moisture level during egg ID
 - Moisten with spray bottle if necessary
- Remove non-target material
 - Look out for eggs
- Finish the paper
- Record rough egg count on paper
- Avoid “overkill”



***Ae. albopictus* eggs**



***Ae. albopictus* eggs, collapsed vs. viable**

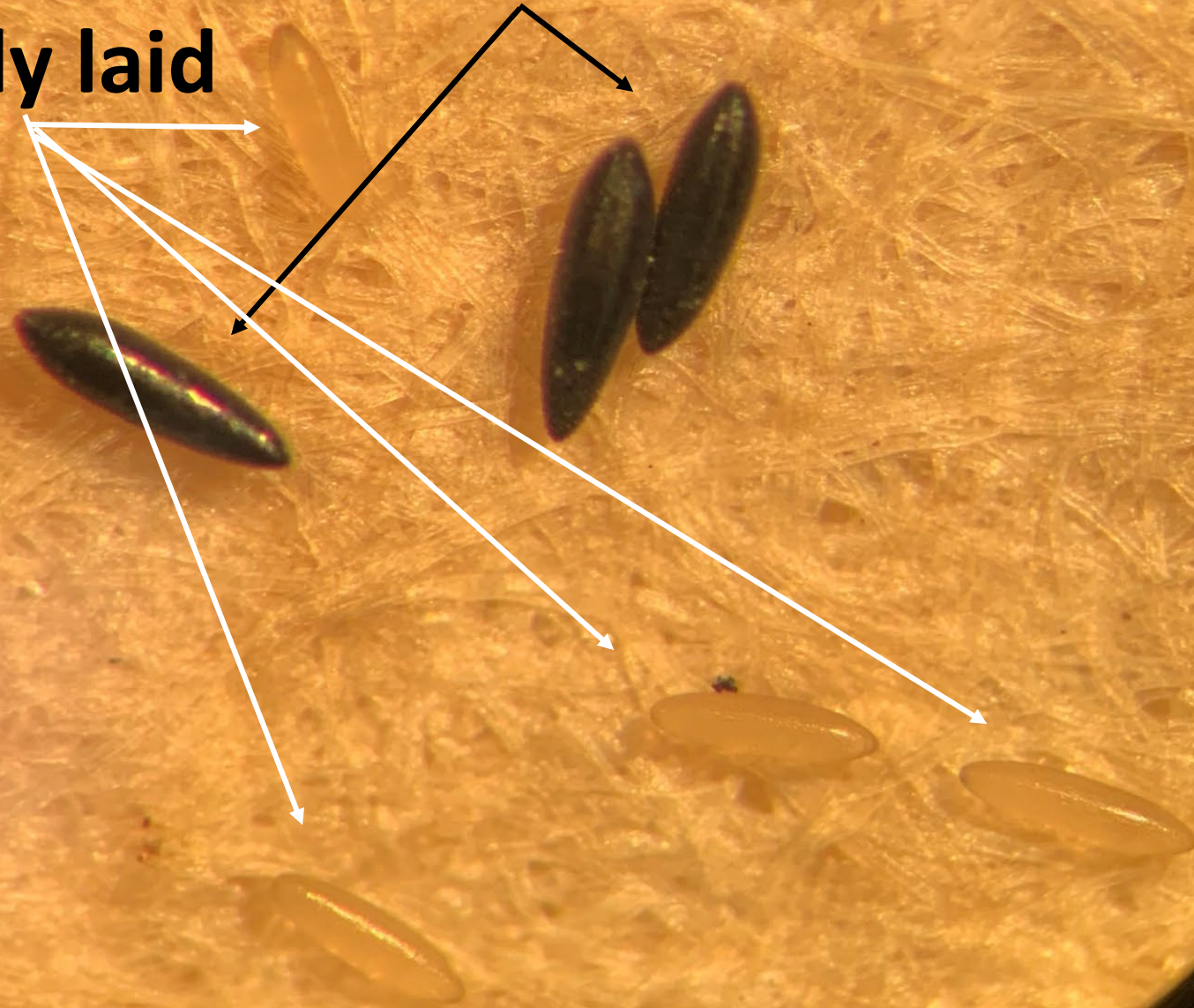


The image shows two larvae of the mosquito species *Aedes albopictus* on a piece of yellowish, fibrous egg paper. The larvae are positioned vertically, one above the other. Each larva has a dark, segmented body and a lighter, segmented head. The background is a uniform, textured yellow color.

***Ae. albopictus*,**
hatched on egg paper



***Ae. albopictus* sclerotized
vs. freshly laid**



***Ae. triseriatus* /
japonicus eggs**



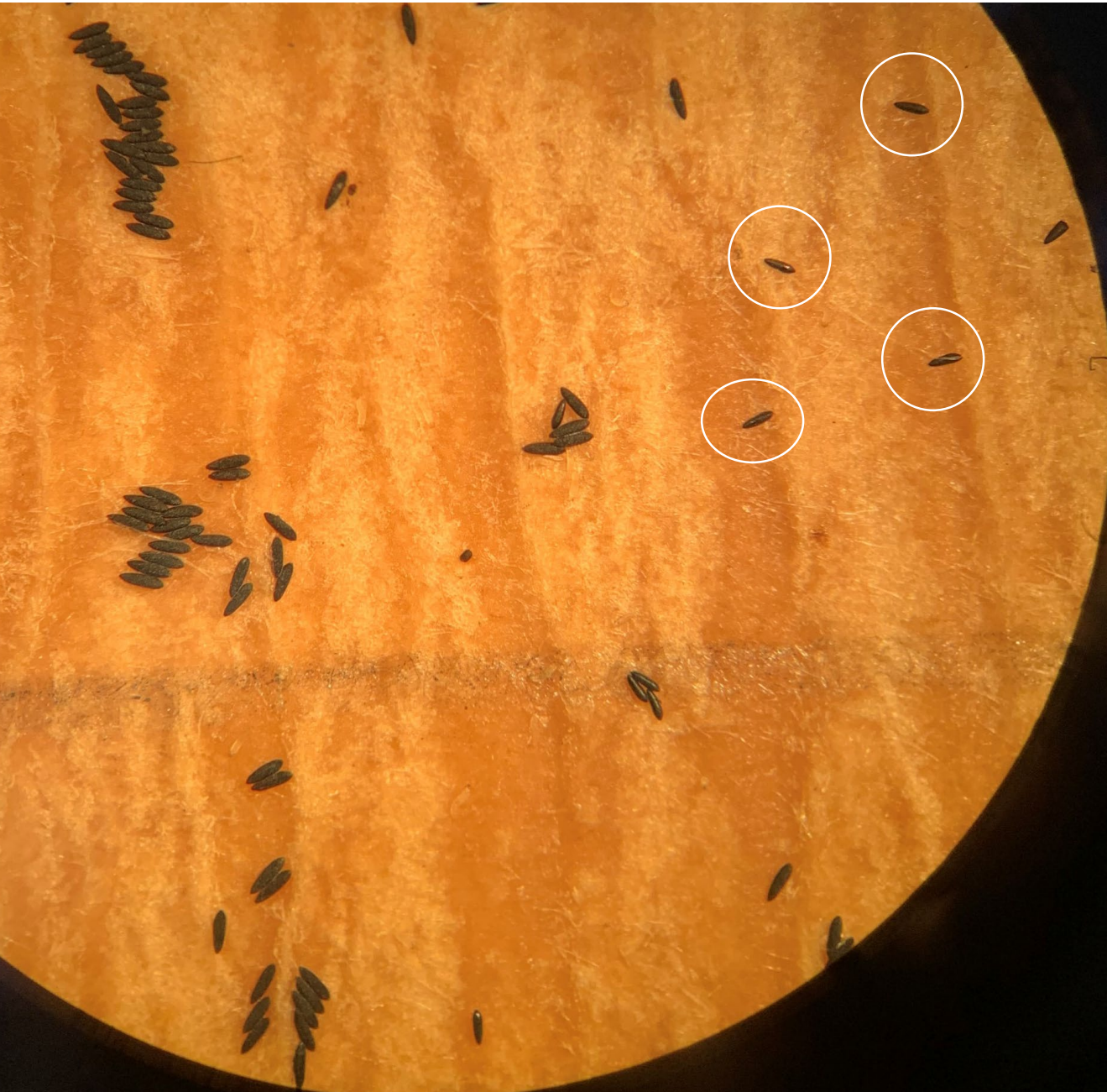
***Ae. japonicus* /
triseriatus stuck to feces**



Ae. albopictus* and *Ae. japonicus/triseriatus



Non-target *Aedes* egg slashing

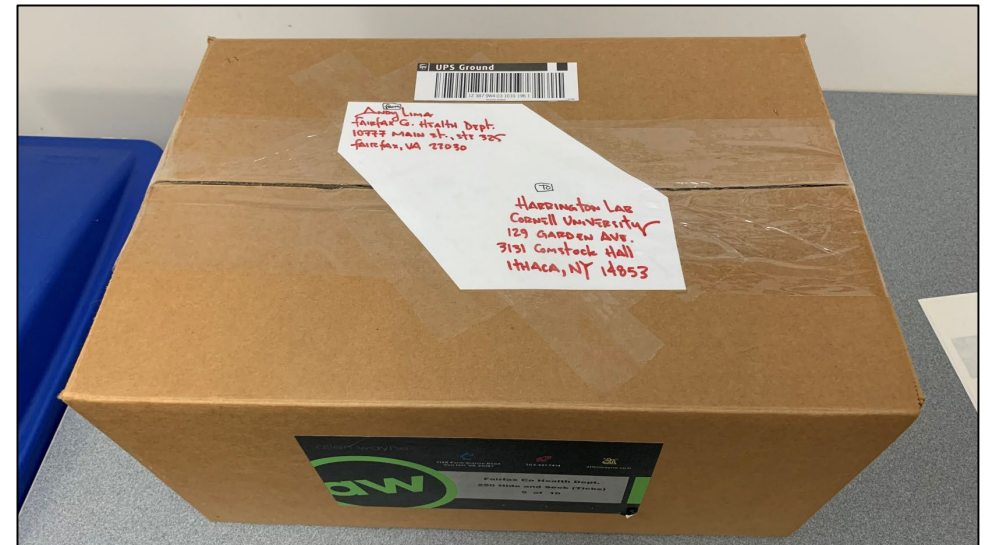
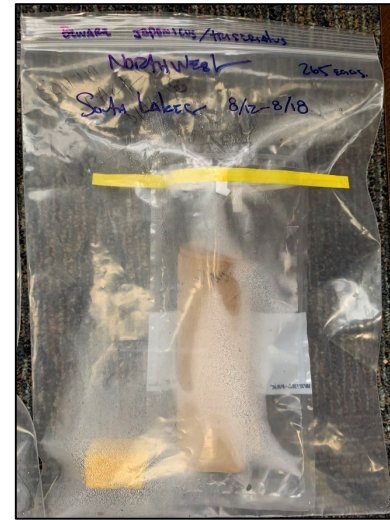






Ae. albopictus shipping: Tips for success

- Monitor dampness of egg papers inside Whirl-Pak / Ziplocks
- Check for mold
- Leave small air pillow inside of Ziplock
- Send with cold packs if not sending overnight
- Stay in touch with receivers





Ae. albopictus egg collections: Challenges / Failures

- Wire wickets
- Unmarked papers
- Over-feeding
- Too much sun
- Rainfall events
- Animal interference
- Human interference
- Swarming
- Premature hatch
- Rapid evaporation



Results

	Resistance Level to Active Ingredients (None, Low, Moderate, High)					
	Etofenprox	Permethrin	Malathion	Methoprene	<i>Bti</i>	<i>Ls</i>
<i>Culex pipiens</i>	High	Moderate	None	Low	None	None
<i>Aedes albopictus</i>	Low	Not tested	Not tested	None	None	Not tested



THANK YOU!

Northeastern Regional Center for Excellence in Vector-Borne Diseases (NEVBD)

Contact us

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NEVBD Pesticide Resistance Monitoring Overview 2022.PDF (Cornell E-Commons)



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Questions?



Photo by Rachel Kempf



Deep thoughts about oviposition

- Why so few egg rafts compared to number of female *Culex* collected the night before?
 - Trapped out?
 - Are we trapping investigatory flights?
- Why do I sometimes see egg rafts on the water after setting a gravid trap?
- Is there a blind spot in my bin?
- What makes a good *Ae. albopictus* ovitrap?
 - Proximity to known larval habitat?
 - Microhabitat within location?
 - Proximity to hosts?
 - The right water?

