

The N.E.V.B.D. Pesticide Resistance Monitoring Program

Specimen Submission System & Services

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Pesticide Resistance Monitoring Program Services



Provide adult & larval resistance testing and supplies

- Egg collection kits
- Larval testing kits

Annual Pesticide Use and Resistance Monitoring Survey

Support establishment of in-house resistance monitoring operations

Advise experimental design and perform customized field trials

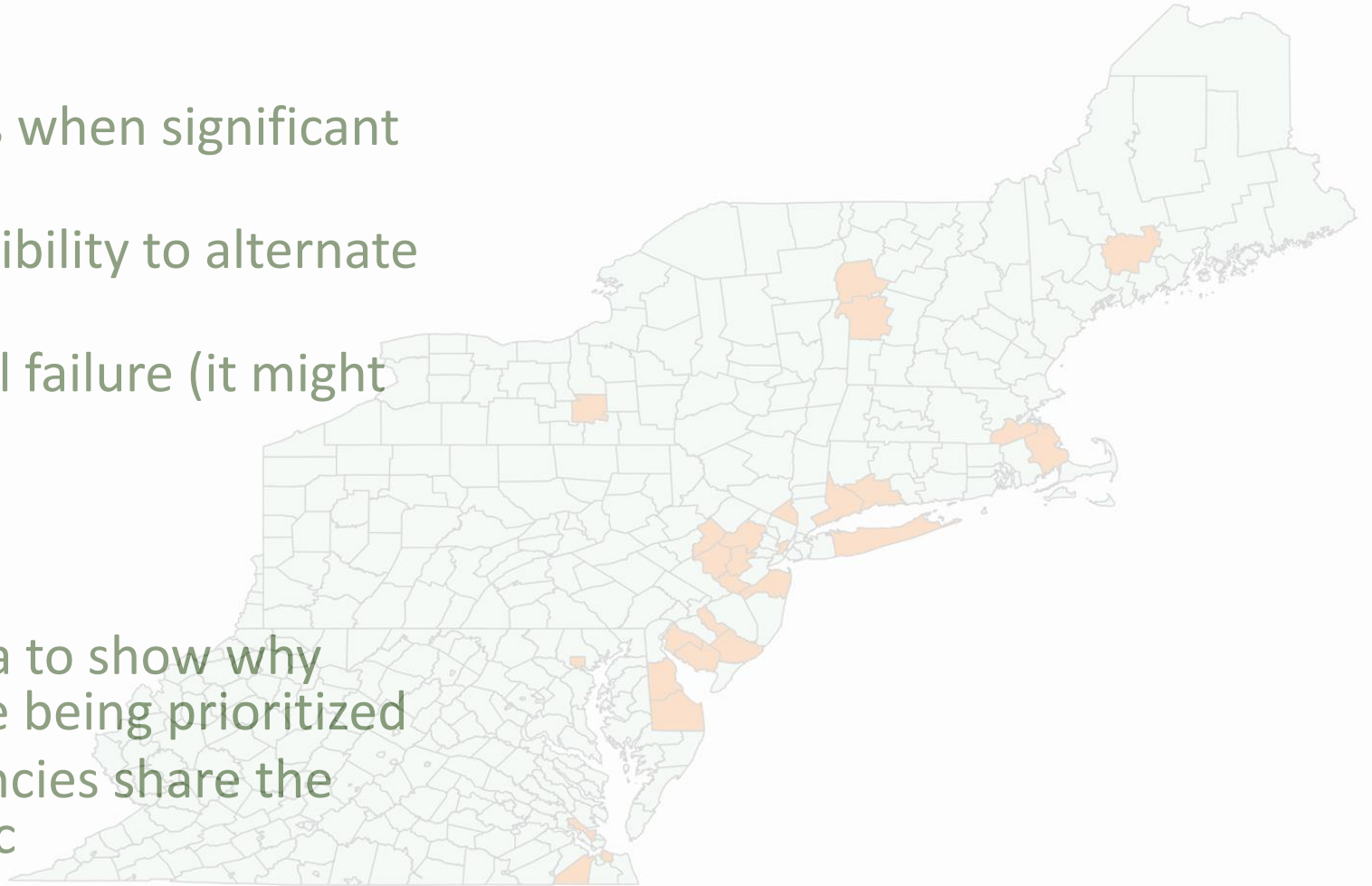
Why participate in the program?

Informs control efforts

- Saves money and resources when significant resistance is detected
- Allows detection of susceptibility to alternate active ingredients
- Can clarify source of control failure (it might not be resistance!)

Communicating with the public

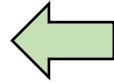
- Helps agencies provide data to show why certain control activities are being prioritized
- Proactively shows that agencies share the same concerns as the public



Testing Services



Photo: CDC bottle bioassay manual



Adult resistance testing

CDC bottle bioassay

Active ingredients

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

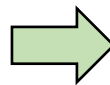
Inhibitors

- PBO - DEF - DM

Larval resistance testing

Active ingredients

- Methoprene - *Bti* – *L sphaericus*

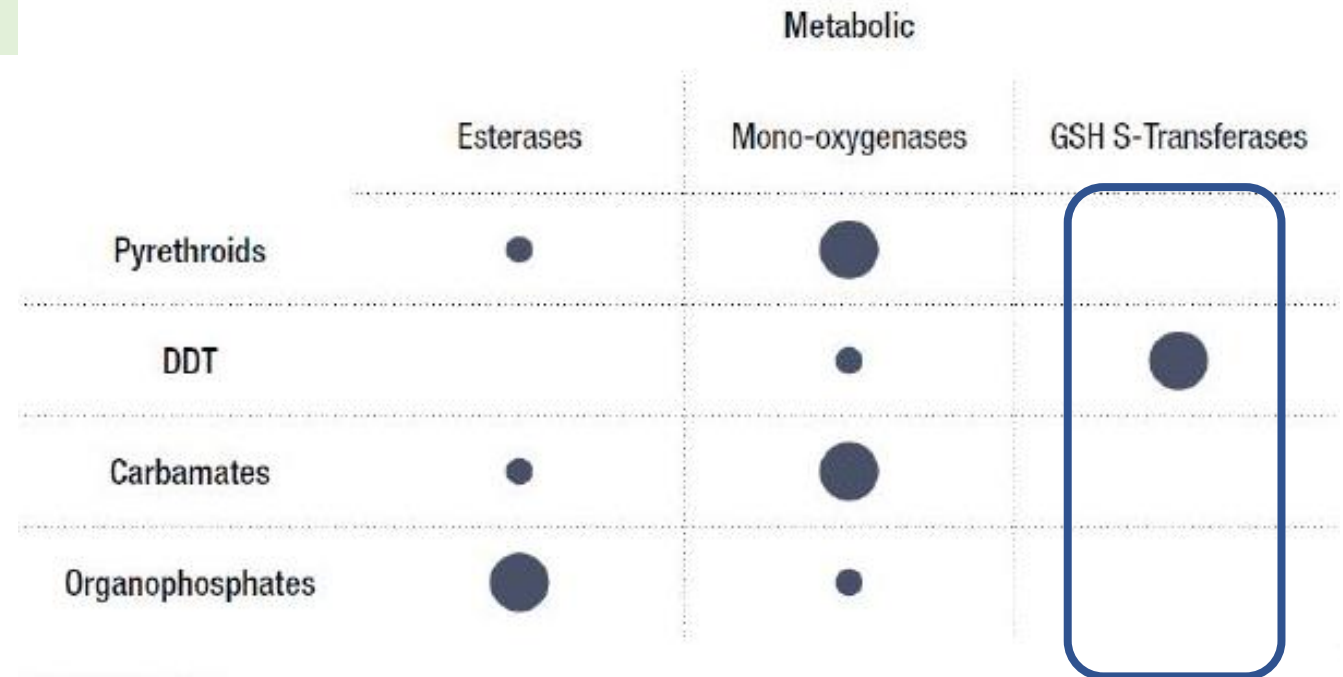


Choosing an Inhibitor (Adult testing)

Inhibitors

- PBO (Piperonyl butoxide) Inhibits oxidase activity
- DEF Inhibits esterase activity
- DM (Diethyl maleate) Inhibits glutathione transferase activity

- Pick an inhibitor based on the class of insecticide
- Test an inhibitor if it is part of a formulation that you use
- Increased mortality with an inhibitor can shed light on mechanism of resistance



From reference (21)

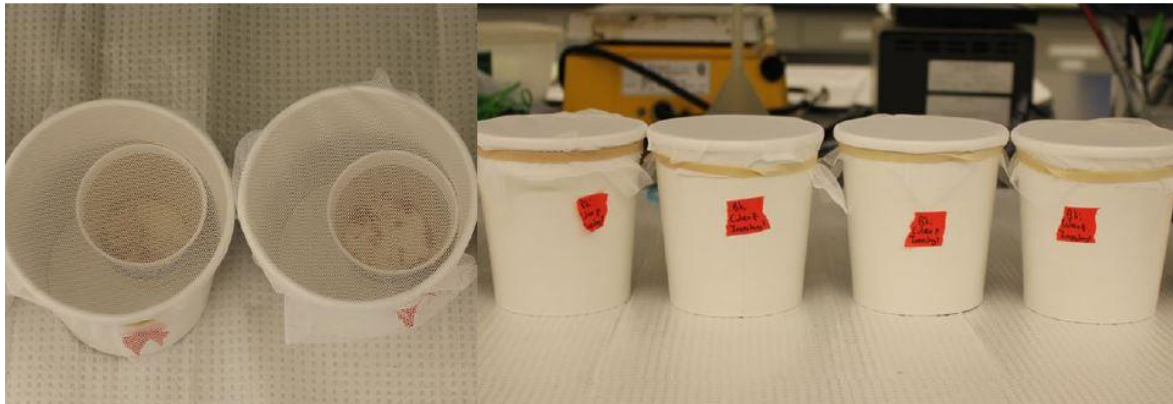
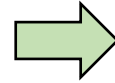
GSH, glutathione; AchE, acetylcholinesterase; circle size reflects relative impact of mechanism of resistance

Kits

Collection kits

Include materials and instructions to collect and submit eggs or larvae for resistance testing

- *Aedes albopictus*
- *Culex pipiens*

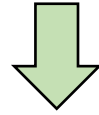


Larvicide efficacy kits

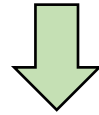
Include materials and instructions to rear larvae and perform in-house larvicide application efficacy testing

Submission system timeline

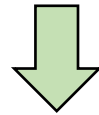
Send out collection kits and instructions each spring



Receive egg and larvae submissions between June and September



Rear mosquitoes in our facility at Cornell University
Perform resistance testing



Report resistance results to submitters and CDC throughout summer and fall

How we collect resistance data

CDC bottle bioassay

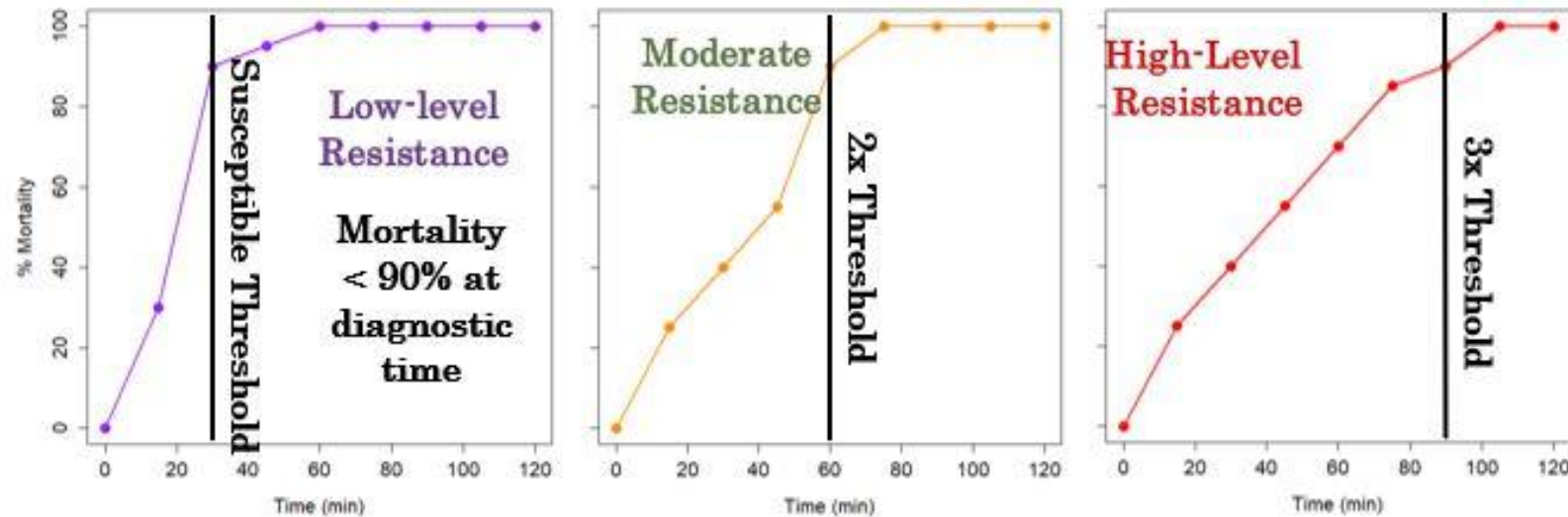
- Rear at least 500 eggs or larvae
 - Adults tested at 3-5 days old
 - Ideally, test 250-275 adult females per bioassay
 - If resistance is found, more individuals will be needed to test with inhibitors
- Resistance is determined by the percentage of mosquitoes that die before the diagnostic time for the active ingredient
- Observed until 100% mortality or up to two hours



How we analyze & report resistance data

The CDC bottle bioassay identifies resistance when there is <90% mortality at the diagnostic time for the active ingredient and species tested

- Level of resistance is defined by percent mortality at 1x, 2x, and 3x the diagnostic time



Reports sent to submitters include species, adulticide, location coordinates, collection date, mortality at the diagnostic and maximum test times, and resistance level detected

Collecting & submitting *Cx pipiens*

Collect *Cx pipiens* eggs July-September

Setting out simple traps near surveillance sites is the best way to consistently collect eggs

- Although egg rafts can be found in industrial areas, human-created containers and catch basins, rain often washes eggs out from breeding sites
- Traps can consist of a bucket filled with dirty water containing hay and poultry waste



Collecting & submitting *Cx pipiens*



Always allow *Culex* larvae to hatch before shipping

- Unhatched egg rafts will break apart, sink, and lose viability when shaken during shipping

Each single egg raft is placed into a falcon tube and allowed to hatch

- *Culex pipiens* must be identified as larvae
- All larvae hatched from one raft can be assumed to be the same species
- We can ID once they arrive

Collecting & submitting *Cx pipiens*

To ship:

- Contents of each falcon tube poured into a separate sample bag
- Pinch of food is added
- Air pocket is left at the top before sealing tightly
- Packed in cooler between layers of paper towel and shipped overnight via Fedex

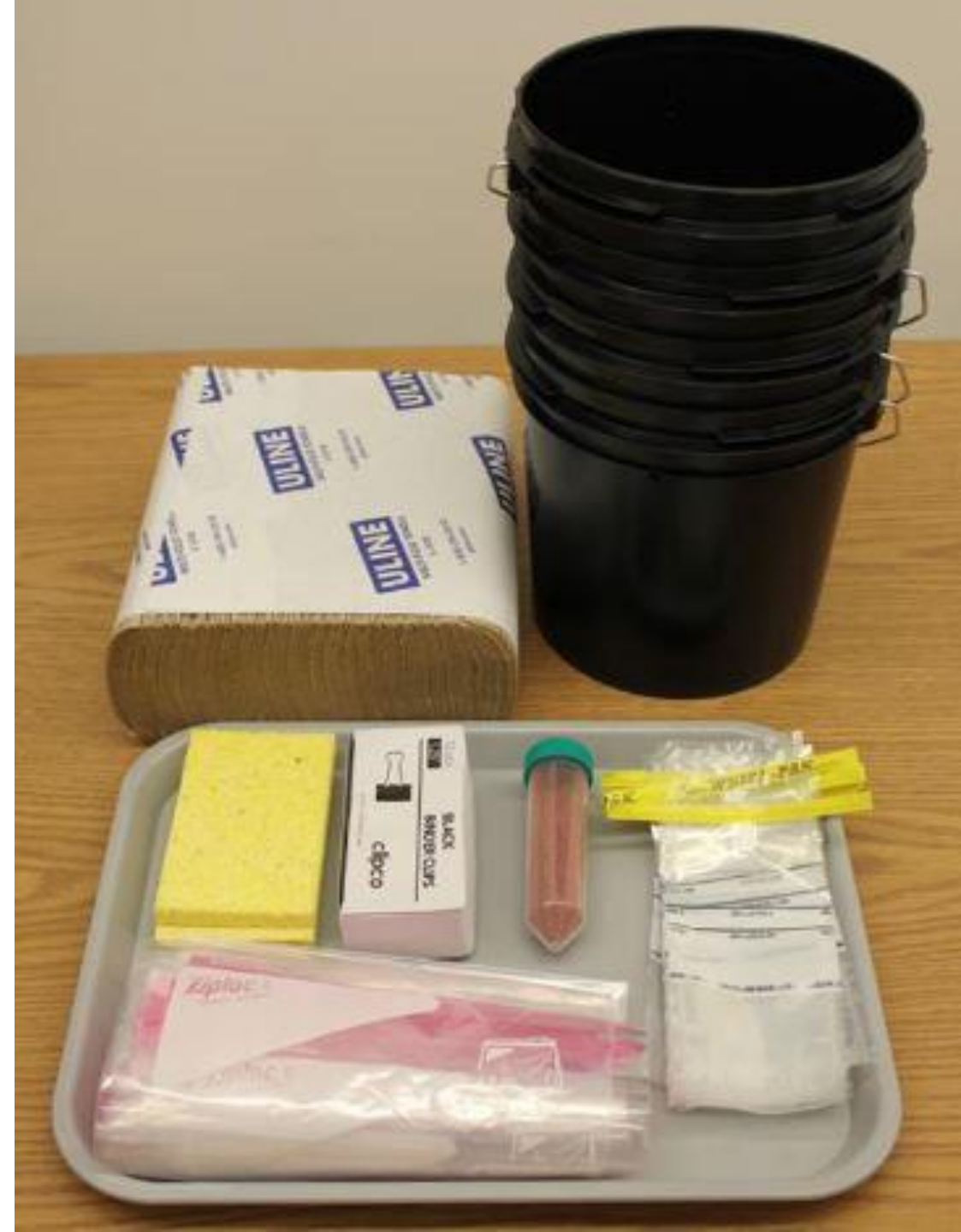


Collecting & submitting *Ae albopictus*

Collect *Ae albopictus* eggs June-August

Placing traps:

- Industrial areas, human created containers and waste locations with community complaints
- Traps should be left for 4-6 days; larvae can hatch if left for too long





Collecting & submitting *Ae albopictus*

We provide an *Aedes* egg identification guide on our website, but we can also identify eggs when they arrive

Ae albopictus eggs can be distinguished by a shiny finish and regular geometry of surface ridges



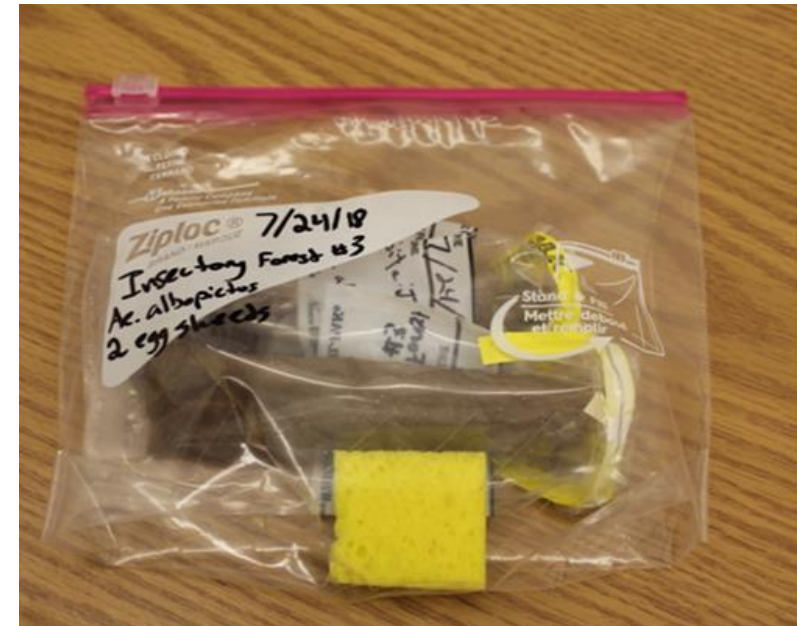
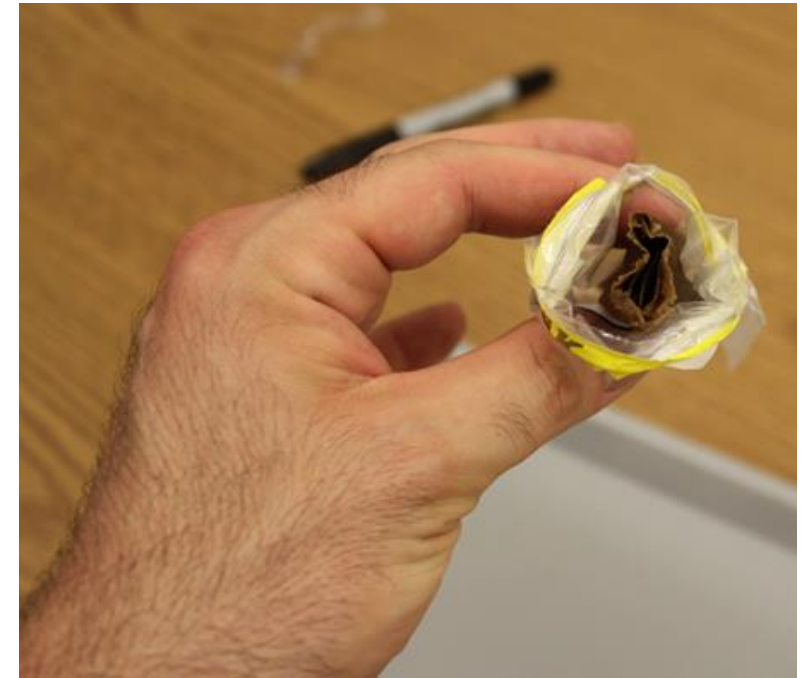
Eggs must go through a drying period for embryos to develop

- Egg sheets are dried flat at room temperature until slightly damp to the touch, but not soaked

Collecting & submitting *Ae albopictus*

1. Egg sheets are placed inside individual open sample bags
2. Sample bags and a damp sponge are placed inside a sealed zipper lock bag

Egg sheets should be shipped as soon as possible after collecting to avoid desiccation



Collecting & submitting tips

Collect from the same fixed location(s) annually

- This allows us to monitor changes in each population's susceptibility to the same active ingredients year after year
- Always record coordinates

Collections can come from existing adult surveillance sites

- Site visits to check traps can be combined with setting out collection materials to save resources

Collecting & submitting tips

Incorporate collecting for resistance submissions into regular surveillance activities

- If already using gravid traps for surveillance, this is a convenient way to collect
- We can perform mechanism testing on the same submission

For general IR surveillance, repeated testing of one location in a single season isn't needed

- Focus on sending enough individuals to test each active ingredient of interest once each year

If funds aren't available to cover shipping costs, contact us



Contact us

General IR program questions or shipment notification: pesticide@cornell.edu

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