Materials Supplied in Kit

This kit contains enough supplies for 20 replicates, but most materials are reusable, if caution is used to avoid contamination. The only exception are the wax-lined cups, which can be purchased at local grocery stores or pharmacies.

Pesticides should be refrigerated for long-term storage.

Item	*Number included in kit*
3 ml Plastic Pipettes	5
Wax-Lined Paper Cups (88 ml / 3 oz)	20
Soup Cups (16 oz)	20
Rubber Bands	20
Fine Mesh Fabric	20
Fish Food	1 Falcon Tube
Falcon Tube (for measuring water)	1

B. Reagents

Methoprene Diagnostic Doses	80 ml (Each species)
Bti Diagnostic Doses (Dilute each 1:500 in H_2O)	10 ml (Each species)

C. Specimens

150 4^{th} instar larvae per site (minimum, testing more improves accuracy)

Materials included in kit

<u>Larvicide Bioassay Procedure</u>*

** The minimum number of containers needed for a sufficient sample size when testing a site for resistance is 10. The first 8 should contain the diagnostic doses of the target pesticide, while only acetone should be added to the last two containers as controls.*

1) Clear a working area of approximately 1.5 ft^2 and lay out 10 of the **wax-lined paper cups** to be used for the trial. Take the pesticides out of the refrigerator so they can come to room temperature.

2) Add a quarter of a **fish food** pellet into each small wax-lined paper cup. In order to measure out a quarter of a pellet, crush it and then the powder into four roughly equal portions.

3) Place one small wax cup with fish food inside each of the **soup cups**. Label two containers as the controls, and the others with the pesticide being tested. Also include the species being tested and any other relevant information (e.g. site name / date) on the label.

4) Using one of the **3 ml plastic pipettes**, count 15 mosquito larvae into the **Falcon Tube**. Once the larvae have been counted, fill the falcon tube to the 45 ml line with water. Pour the water and larvae into the smaller cup and then add an additional 30 ml to cup so that they each contain a total of 75 ml of water.

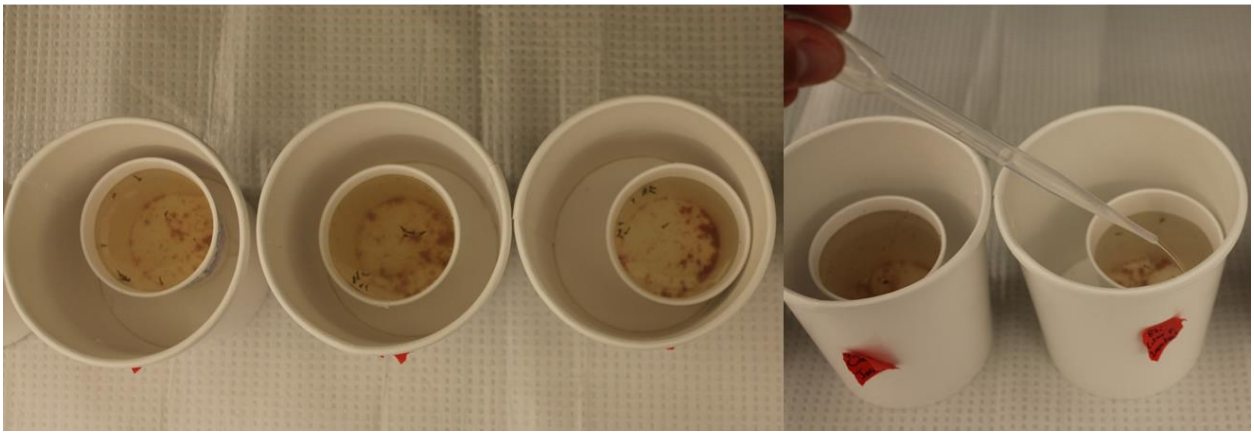
5) Use the plastic pipette to remove 1 ml of water for methoprene trials or 5 ml for Bti trials.

6) Adding pesticides (Diagnostic concentrations listed in table A1)

   a. METHOPRENE TRIALS: Gently shake the vial containing methoprene for at least 30 seconds then add 1 ml of the diagnostic concentration for the target species to each small wax-lined cup, except the two control cups. Add 1 ml of pure acetone to the control cups. (Nail polish remover or other acetone product can be used here, if analytical-grade acetone is not available.) ***Use separate pipettors to add the control (acetone) and methoprene to avoid contamination.***

   b. Bti TRIALS: Gently shake the vial containing Bti for at least 30 seconds. Make a 1:500 dilution of the Bti in water (1 ml of Bti solution + 499 ml of water). The falcon tube can be used to measure 499 ml of water and the pipettor can be used to add the 1 ml of Bti. This dilution can be stored for one week before being discarded. Add 5 ml of the diluted Bti for the target species to each small wax-lined cup, except the two control cups. Add 5 ml of water to the control cups for the Bti trials. ***Use separate pipettors to add the control (water) and Bti to avoid contamination.***

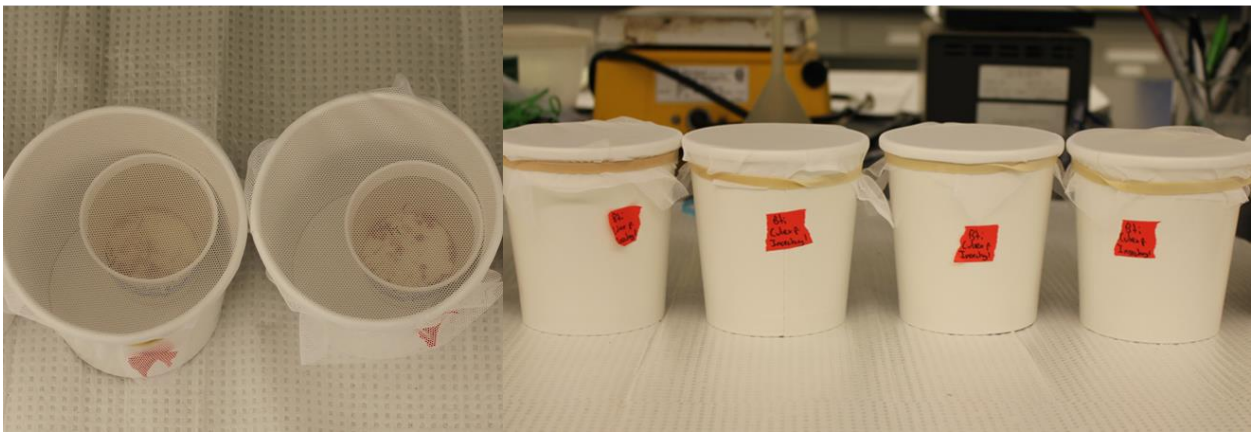
(A) **(B)**



(A) Once the larvae and water have been poured into all the assay replicates, (B) add the appropriate volume of methoprene (1 ml) or Bti (5 ml) to all, but the control cups.

7) Use the **rubber bands** to attach the **fine mesh fabric** to the top of the soup cups to prevent adults from escaping. Place the containers on a table away from sunlight in a temperature-controlled room.

(A) **(B)**



(A) The cups should be covered in mesh (B) by carefully applying a rubber band. Be sure to cover the top completely so no adults can escape, particularly for the methoprene trials

8) Collecting data

   a. <u>METHOPRENE TRIALS</u>: Check the containers every 24 hours for the next 6 days and record the number of adults that have emerged in each container. If more than 5% of adults have emerged this may indicate emerging resistance in the sampled population.

   b. <u>Bti TRIALS</u>: Count and record the number of larvae alive after 24 hours. If more than 5% of larvae are alive this may indicate emerging resistance in the sampled population.

9) After the trial is over, discard the small cups, the rest of the materials can be reused.

10) If mortality in the control cup is > 20% discard this trial and start again.

Appendices

Table A1: These are the diagnostic doses to be used based upon susceptibility curves constructed with susceptible strains for *Culex pipiens* and *Aedes albopictus* at 28 °C and 80 % -to- 90 % relative humidity. These values represent the concentrations after addition to the 75 ml of water, so the concentration in the vials included in the kits are higher, but simply need to be added to the water. Please contact James Burtis (jb766@cornell.edu) for further information about the susceptible colonies used.

Species	**Methoprene Diagnostic concentration (2x LC-99)**
Aedes albopictus	0.171 µg / ml
Culex pipiens	0.026 ug / ml
Species	**Bti Diagnostic concentration (2x LC-99)**
Aedes albopictus	0.117 ITU / ml
Culex pipiens	0.030 ITU / ml