IRAC Susceptibility Test Methods Series
Version: 1.0

Method No: 028

Details:

Method:	028
Status:	Approved
Species:	Stink Bugs – Bean Dip Assay (Hemiptera: Pentatomidae) Validated for: *Euschistus heros*
Species Stage	Adult
Product Class:	Pyrethroids (IRAC MoA 3A) Neonicotinoids (IRAC MoA 4A)
Comments: None	

Euschistus heros
Photograph Courtesy of:
J.J. Silva

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:

Aerated insect-proof containers, forceps or brush for transferring insects, Petri dishes (100 mm x 15 mm), filter paper (70-90 mm), syringes/ micropipettes for liquids, beakers for formulating solutions, paper towels, fresh common green bean pods (*Phaseolus vulgaris*), knife for cutting beans, seeds (soybean, peanut, and sunflower), chlorine bleach, maximum/ minimum thermometer

Methods:

a) Collect adult stink bugs from multiple random locations within an infested field. Store insects in aerated insect-proof containers. Ensure that the insects are not subjected to excessive stress after collection (temperature, humidity, starvation, etc.). Transfer insects to laboratory as soon as possible.

b) After arriving in the lab, allow the insects to recover overnight prior to testing. The stink bugs can be maintained on a diet consisting of fresh green bean pods (*P. vulgaris*), and a mixture of soybean, peanut, and sunflower seeds (Figure 1).

c) Prepare the test arena by placing a sheet of filter paper in a Petri dish and moistening the paper with 1 ml distilled water.

d) Transfer 5 adult stink bugs into each Petri dish (Figure 2). Each plate is considered one plot. Replicate each plot four times for each concentration of the insecticide. Prepare four additional replicates for untreated controls.

e) Prior to testing, wash fresh bean pods (*P. vulgaris*) in 1% chlorine bleach solution and allow to dry. Cut each pod into 2-3 pieces (~ 4-5 cm long).

f) Prepare appropriate test dilutions in water; the use of a wetter is not necessary. Select a series of concentrations to give a range of mortality for a clear dose response for the insecticide(s) being evaluated. At least 5-6 concentrations are recommended. A final volume of 100 ml should be sufficient for this assay. Create an untreated control solution similar to the treated solutions but without any insecticide.

g) Agitate the treatment solution and completely dip the bean pod pieces in the solution. Keep the bean segment submerged in the solution for 3-5 seconds. Place the treated bean pods onto paper towels and allow to dry (Figure 3).

h) Place 3 treated bean pod pieces in each Petri dish plot. Store Petri dishes in area where they will not be exposed to temperature extremes (Figure 4). Record maximum and minimum temperatures.

i) Assess mortality 96 hours after application. Count number of affected (dead and moribund) insects. Correct for untreated control mortality using Abbott's formula. Use the corrected mortality data to perform a logistic or probit dose response analysis to estimate LC_{50} or LC_{90}. If mortality in the untreated control treatment exceeds 20%, the study should be considered invalid for the purpose of resistance monitoring.

Figure 1. Stink bugs maintained on bean pods and seeds in the lab (photo courtesy BASF).

Figure 2. Petri dish test arena with moistened filter paper (photo courtesy BASF)

Figure 3. Treated bean pod pieces drying on paper towels (photo courtesy BASF).

Figure 4. Test in progress (photo courtesy BASF).

Precautions & Notes:
None

References & Acknowledgements:
None