

## IRAC Susceptibility Test Methods Series *Version: 1.0*

Method No: 029

### **Details:**

Method:	029	
Status:	Approved	
Species:	Stink Bugs – Topical Assay (Hemiptera: Pentatomidae) Validated for: <i>Euschistus heros</i>	
Species Stage	Adult	Cores )
Product Class:	Pyrethroids (IRAC MoA 3A) Neonicotinoids (IRAC MoA 4A)	Euschistus heros Photograph Courtesy of: J.J. Silva
Comments: None		

#### **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 and topical applications, 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) 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).
- d) Prepare the test arena by placing a sheet of filter paper in a Petri dish and moistening the paper with 1 ml distilled water (Figure 2). Place 3 cut bean pods into each dish as a food source.
- e) Transfer 5 adult stink bugs into each Petri dish. Each dish is considered one plot. Replicate each plot four times for each concentration of the insecticide. Prepare 4 additional replicates for the untreated controls.



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- f) Select a series of concentrations to give a range of mortality for a clear dose response for the insecticide(s) being evaluated. Prepare appropriate test dilutions in water, at least 5-6 concentrations are recommended. Create an untreated control solution similar to the treated solutions but without any insecticide.
- g) Apply 1 drop (2 μl) of test solution to the back (dorsum) of each insect using a micropipette. Store Petri dishes in area where they will not be exposed to temperature extremes (Figure 3). Record maximum and minimum temperatures.
- h) Assess mortality 72 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<sub>50</sub> or LC<sub>90</sub>. 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).



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Figure 3. Applying test compounds to the dorsum of the insect (photo courtesy BASF).

Precautions & Notes: None

References & Acknowledgements: None