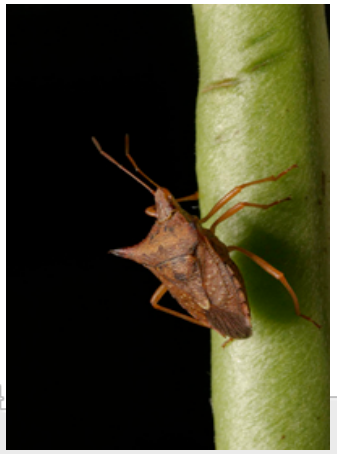
IRAC Susceptibility Test Methods Series
Version: 1.2

Method No: 030

Details:

Method:	No: 030	 *Euschistus heros* *Photograph Courtesy of: J.J. Silva*
Status:	Approved	
Species:	Stink Bugs – Vial Assay (Hemiptera: Pentatomidae) Validated for: *Euschistus heros*	
Species Stage	Adults	
Product Class:	Carbamates (IRAC MoA 1A) Organophosphates (IRAC MoA 1B) Pyrethroids (IRAC MoA 3A) Neonicotinoids (IRAC MoA 4A)	
Comments:		

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:

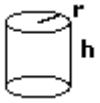
Aerated insect-proof containers, forceps or brush for transferring insects, glass beakers for test liquids, glass vials (approx. 100-150 ml volume) with lids, vial roller (or hotdog roller), acetone, pipette for liquid or weighing balance for solid products, ventilated holding cage, maximum/minimum thermometer.

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 the laboratory as soon as possible.
NOTE: Use the attached recording sheet for sampling details and other information that may be useful for tracking samples and interpreting susceptibility results later on.

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) The test containers are glass vials with an internal surface area of 100-150 cm^2.
Determine the surface area of the glass vials by:
(h is the height of the vial, r is the radius of the bottom)



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Surface Area = Area of bottom + Area of the side
Surface Area = $\pi r^2 + (2 \pi r)* h$

d) Prepare dilutions of the technical grade compound in acetone. Glass vials should be filled with 500-1500 µl of test solution to evenly coat the entire internal surface area (solution should cover the base of the vial, with spilling out of the open neck of the vial when laid on its side). Rotate open treated vials on a vial/hotdog roller at room temperature until the acetone is completely evaporated (Ensure all acetone vapor is eliminated).
A minimum of three replicates of each concentration and control are required, but 4-6 replicates are recommended. Use an acetone-only treatment as a solvent blank.

e) **Dose response:** Prepare appropriate test dilutions in acetone. Select a series of concentrations to give a range of mortality for a clear dose response for the insecticide(s) being evaluated. At least 3 doses should provide a range of mortality between 30% and 95% in order to be able to provide an accurate dose response curve.

Discriminating doses: Discriminating doses can provide a more resource favorable way of surveying insect susceptibility in the field. Prior to determining appropriate discriminating doses it is important to determine the natural variations in insect susceptibility to the insecticide (baseline susceptibility). It is recommended to test 10-20 populations of the target insect collected from variously locations using dose response assays.

Discriminating doses are often selected on a fixed susceptibility value such as the concentration of the insecticide which provides 90%, 95% or 99% mortality and multiplications of this value, such as 10x the dose that provides 90% mortality. It is important to note that survivorship at these dose does not necessarily indicate resistance and natural variation in susceptibility should also be considered.

Alternatively, a dose which is considered to be the equivalent of the recommended field rate and multiples of this rate are sometimes used as discriminating doses (e.g. 200%, 100% and 20% of the typical field application rate). Field rates (g active ingredient (a.i./ha) can be converted to µg/cm² by simply multiplying by 0.01. However researchers should consider that exposure to a technical insecticide on a glass surface does not necessarily represent the same level of insecticide exposure that an insect may experience whilst inhabiting plant material treated with a commercial formulation of the same insecticide.

NOTE: Field rates will likely be different for each compound being tested, when preparing test solutions, refer to the labeled rate for the compound being tested. Additional rates are required if a full dose response for the generation of accurate susceptibility data (LC50 values) is desired.

f) Place at least five adult stink bugs per vial, cap and store at 20 ± 5ºC. Avoiding exposure to an uneven light source or direct sunlight. Physical contact with the insects should be kept to a minimum. Placing large numbers of insects in the glass vial can lead to 'piggy-backing' of the insects to avoid insecticide deposits and providing variability in the test system. Avoid this by not placing too many insects in each vial.

Stink bug nymphs can also be tested using the same system, however please note that the susceptibility of the different life stages may be different and therefore a discriminating dose which has been developed for one life stage may not be appropriate for other life stages.

g) The number of stink bugs severely affected (dead and moribund) is scored after a 24 hour period for pyrethroids and neonicotinoids. A 48 hour period is recommended for organophosphates and carbamates. The assessment can be made visually through the vial or by emptying the bugs from the glass vial onto the centre of a piece of paper with a 15cm circle drawn in the middle. The assessment

should be made in bright light to stimulate stink bug movement out of the circle and the bugs which cannot exit the circle before a period of one minute should be considered severely affected.

h) Express results as percentage affected. If greater than 20% of the control treatment is severely affected, then the study should be considered as invalid for the purposes of resistance monitoring.

Expect a degree of natural variability in biological results. This expected level of natural variability will be indicated in the baseline susceptibility survey. If baseline susceptibility data is not available then populations of insects which are suspected of being resistant should be compared to a field collected strain of the insects which has had minimal insecticide exposure (field susceptible) or less preferably a laboratory susceptible strain.

Precautions & Notes:

a) Where glass equipment is used, it must be adequately cleaned with an appropriate organic solvent before re-use to prevent cross-contamination.

b) Different batches of technical grade insecticide may vary in concentration of active ingredient (usually between 85-99% a.i.). It is recommended to use high purity a.i. where possible. Purity needs to be taken into account when preparing the test solutions.

c) The use of formulated products should be avoided as their reaction to being diluted in acetone cannot be predicted and changes in formulation types between regions and years can lead to problems in data comparison.

References & Acknowledgements:

None

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

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Sample Information Recording Sheet:

Sampling details	
Susceptibility problem previously apparent:	Yes/no
Date of insect collection:	
Address:	
Name of collector	
Street	
Postal code	
City	
Region	
Geographical position (GPS), if available:	
Crop:	
Average number of insecticide applications in the region:	
Recent insecticide applications in the sampled field:	
Product	
Application date	
Number of applications	

For further information please contact the IRAC International Coordinator:
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