


## IRAC Susceptibility Test Methods Series Version: 1.0

Method No: 033

### Details:

Method:	No: 033	 <p><i>Lygus hesperus</i> Photograph courtesy of: Bayer CropScience</p>
Status:	Approved	
Species:	<i>Lygus hesperus</i>	
Species Stage	Adults or nymphs	
Product Class:	Pyrethroids (3A), Carbamates (1A), Organophosphates (1B), nAChR agonists (4)	
Comments:		

### Objectives:

Susceptibility Baseline:

Resistance Monitoring:

### Description:

#### Materials:

Aerated insect-proof containers, aspirator (200 µl pipette tip with tips cut for *Lygus* to pass safely) or paintbrush for transferring insects, glass beakers for test liquids, pipette for liquid or weighing balance for solid products, acetone, bleach solution (e.g. Clorox<sup>®</sup>), and petri dishes (100x100x10 mm, or 100 mm diameter).

#### Methods:

- Collect insects from multiple random locations within an infested field, and keep them in an aerated insect-proof container. Ensure that insects are not subjected to excessive stress after collection (temperature, humidity, starvation, etc.). Transfer insects to the laboratory as soon as possible. Record sample details and other information that may be useful for tracking samples and interpreting susceptibility results later on (a sample records form is provided below).
- After arriving in the lab, allow the insects to recover overnight prior to testing. *Lygus* bugs can be maintained on the plants that they were collected from or on fresh green beans (washed with a 1% bleach solution to remove any residual insecticides). Select uniform individuals/life stages for testing (e.g. nymphs or adults).
- Purchase fresh green beans, wash the beans in a 1% bleach solution, triple rinse with deionized (DI) water, and allow to air dry for at least 30 minutes.
- Prepare appropriate test dilutions of formulated products in DI water containing 0.01% Kinetic<sup>™</sup>, or technical grade active ingredients (AI) in 50:50 acetone: water containing 0.01% kinetic. Ensure that the product is completely dissolved. Select a series of concentrations (4-6 rates) to give a range of

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mortality for a clear concentration response for the insecticide(s) being evaluated.

- e) Shake the test solutions and then dip the washed beans individually for ~3 s, and allow to air dry for at least 30 min. For best results, use whole green beans.

Place the dried treated beans in petri dishes individually in preparation for infestation. Replicate each treatment at least 6 times (petri dishes) per treatment.

- f) Infest each petri dish with 3-4 individuals (Figure 1). Insects can be transferred using an aspirator or they can be anesthetized with CO<sub>2</sub> and transferred with a paintbrush.
- g) Following infestation, all dishes should be arranged randomly and kept at 27±2°C on counter top or shelves.
- h) Assess mortality between 3-5 days after infestation (test length may vary depending on the AIs being evaluated). Insects unable to right themselves when probed should be considered dead.
- i) Express results as a percentage mortality. Correct for 'untreated' (control) mortalities using Abbott's formula (Abbott 1925). The mortality data can be subjected to a probit or logit dose response analysis to calculate an LC<sub>50</sub> or LC<sub>90</sub>.
- j) If mortality is greater than 20% for the control treatment, the study should be considered as invalid.

### **Precautions & Notes:**

- a) Where glass equipment is used, it must be adequately cleaned with an appropriate organic solvent and/or lab detergents 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% AI). It is recommended to use high purity AI where possible. Purity needs to be taken into account when preparing the test solutions.

### **References & Acknowledgements:**

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

This methodology was provided by BASF

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**Figure 1.** Treated green bean infested with *Lygus* (photo courtesy BASF).

### 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	