IRAC Susceptibility Test Methods Series

Method No: 017

Version: 3.9

Details:

Method:	IRAC No. 017
Status:	Approved
Species:	Codling Moth *(Cydia pomonella)*
Species Stage	Larvae (L1)
Product Class:	This method is specifically recommended by the IRAC Diamide Working Group for evaluating the susceptibility status of **diamide insecticides (IRAC MoA 28)*.** This method is also suitable for the following insecticide classes (IRAC MoA class): Organophosphate (1B)* Pyrethroid (3A)* Neonicotinoids (4A)* Spinosyn (5)* Avermectin (6)* Juvenile Hormone Mimics (7A)** Fenoxycarb (7B)** Benzyl urea (15)** Diacylhydrazine (18)** Indoxacarb (22A)* Metaflumizone (22B)* Pyridalyl (un)*

Cydia pomonella larvae and damage
Photographs Courtesy of:
DuPont Crop Protection

Comments:

Mortality assessment period may vary depending on insecticide mode of action

The following guidelines may be used:

*96-hour assessment period
**120-hour assessment period (Larvae should go through full molt before mortality assessment).

For the purposes of this methodology the density of water is assumed to be 1.00g/ml

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

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Description:

Materials:

Distilled water, mixing bowl, weighing scales (0.001 g accuracy),syringes/pipettes and beakers/test tubes for making dilutions, artificial diet (Stonefly *Heliothis* Premix Diet Formula)*, 10-20 mm diameter well plates with sealable lid**, protective gloves, fine artist's paintbrush, fine forceps or seeker, binocular microscope or hand lens (optional), maximum/minimum thermometer.

*Available from Ward's Natural Science Establishment, LLC (www.wardsci.com)
**Bio-Serv, 128 cell bio-assay tray is recommended: Product code : BAW128 (www.insectrearing.com)

Method:

(a) Collect a representative sample of insects from a field. These may be larvae, pupae or adults for rearing to the appropriate stage or material from which an F1 population for testing can be reared. The insects should not be subjected to temperature, humidity or starvation stress after collection. A minimum of 100 larvae or diapausing pupae should be collected for each population to be tested, in order to establish a breeding colony of at least 50 adults.

(b) Prepare accurate dilutions of the test compound from the identified commercial product. Ensure thoroughly mixing at each dilution step. Six evenly spaced rates allowing to get a clear dose response are recommended.

(c) A half milliliter (0.5 ml) of each insecticide solution is required per 20 g of diet to be prepared. Weigh and add 20 g of Stonefly *Heliothis* Premix Diet to the glass bowl and thoroughly mix all the ingredients together until soft, smooth dough is obtained. Ensure protective gloves are worn during this procedure to avoid exposure to the insecticide.

(d) Add 0.5 g pieces of the dough to individual wells of a 10-20 mm diameter well plate, press the dough gently and evenly to fill the base of each well. A minimum of 40 wells per insecticide concentration should be prepared.

(e) Repeat steps (c) & (d) for each of the insecticide concentrations utilized in the study and a control treatment, which should utilize distilled water only for step (c) instead of insecticide solution. Ensure that mixing equipment is cleaned and protective gloves are replaced between treatments to prevent contamination.

(f) Add a single neonate (less than 24 hour old) of *Cydia pomonella* larvae to each individual test well using a fine artist's paintbrush. Once a plate is fully infested, seal tightly with the lid (ensure no gap between the top of the plate and the lid) to prevent larvae from moving out of their assigned well.

(g) Store the plates under 22±2°C, 60% RH and 16:8 light/dark regime is preferred. If not possible, avoid direct sunlight or extremes of temperature and record maximum and minimum temperatures.

(h) In the case of diamide insecticides, a final assessment of larval mortalities (dead and live) is made after 96 hours. For other insecticides please see guidelines provided at the top of this document.

(i) The number of dead larvae and moribund larvae (seriously affected larvae which are unable to make coordinated movement and cannot return to an upright position when turned upon their backs with a seeking pin or fine pointed forceps) are to be summed and considered as dead. Untreated mortality should be recorded. Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. It is recommended that the

corrected mortality data is utilised to perform a probit or logit dose response analysis to provide LC50 and LC90 estimates for each insecticide or insect population tested.

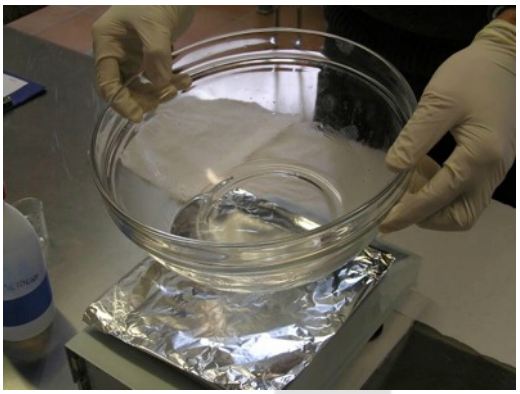

Fig 1: Measuring solutions

Fig 2: Mixing of stonefly diet and solutions

Fig 3: Fully mixed diet

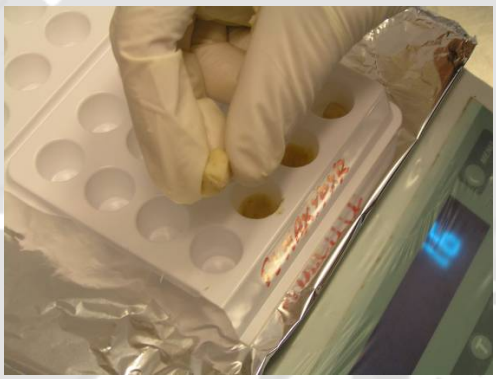

Fig 4: Adding diet to individual wells

Precautions & Notes:

1. Disposable plastic equipment is preferred provided that it is not affected by the formulation constituents; glass equipment may be used but must be adequately cleaned with an appropriate organic solvent before re-use.

2. Insecticide products contain varied concentrations of active ingredient(s). Ensure insecticide dilutions are based on active ingredient content (g a.i.). Some diamide insecticides are sold as pre-mixtures with other insecticides; these products should not be used to determine the susceptibility of insect populations to the single insecticide, as the mixture partner may have a significant impact on the mortality data generated.

3. Where possible, bioassays to measure the variation in insecticide susceptibility of field populations should run in parallel with a known susceptible standard population of the insect.

References & Acknowledgements:

This IRAC method is based on a method developed by Luigi Caroli (Agronomica RS Terremesse, Ravenna, Italy), Greg Krawczyk (Pennsylvania State University, Biglerville, USA) and Jay Brunner/ Mike Doerr (Washington State University, Wenatchee, USA). Photographs are courtesy of Agronomica RS Terremesse.