IRAC Susceptibility Test Methods Series

Version: 1.0 (7th February 2023) *Method No: 034*

Details:

Method:	No: 034	 *Scirpophaga incertulas* larva Photograph courtesy of FMC
Status:	Approved	
Species:	*Chilo suppressalis* *Scirpophaga incertulas* *Scirpophaga innotata* *Sesamia inferens*	
Species Stage	Second instar (L2) or third instar (L3)	
Product Class:	Indoxacarb (MoA 22) Diamides (MoA 28)	
Comments: This method has only been approved and validated for species and active ingredients indicated. This method was validated for *S. incertulas* and *S. inferens* with MoA 22. It was validated for *C. suppressalis*, *S. incertulas*, *S. innotata*, and *S. inferens* with MoA 28. The method may be applicable for other MoA, but further validation is required and ongoing.		

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:

- Mylar cage (10 cm diameter X 30 cm height) or fabric bag for adult moths during oviposition
- Gauze fabric to cover cages, and rubber bands or twine to secure
- Formalin to sterilize eggs
- 500 mL cup to germinate rice seeds for rearing caterpillars to 2nd instar
- 20 mL glass cannon vials to hold egg masses for hatching
- Scissors to cut tillers, stems, and leaves
- Absorbent paper or cotton to maintain humidity in vials
- Petri dishes and filter paper for hatching eggs (14 cm) and assay containers (9 cm)
- Rice seed, pots (10 cm), soil for growing rice plants
- Aerated holding cages to protect from predation, rearing with use of potted plants
- Balance for weighing chemicals, pipette & tips for dilutions, non-ionic surfactant
- Glass or disposable plastic beakers (500 mL) for mixtures to dip plants or tillers
- Paintbrush or forceps to transfer insects to assay containers
- Rearing trays (16-32 cell) and plastic or sticky lids (4 cells) for assays using cut tillers
- Agar or absorbent fiber squares to maintain humidity in assay tray cells
- Aluminum foil as a drying surface after tillers are dipped with pesticide treatments

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Methods:

Test Plants

a) Use untreated *Oryza sativa* L. (rice) varieties that are susceptible to stem borer (e.g., 'Taichung Native 1' or 'IR-22').

Field Collection

b) Per population, collect at least 100 adult moths across multiple areas of a large field. Collection is easier at night directly from plants, near streetlights, or with moth light trap.

- Egg masses can be collected directly from the field but are prone to microbial contamination, therefore it is not recommended.

c) Identify species. Be aware that development time may differ for each species.

Insect Production

d) Partition moths into four ovipositional cages.

- Cage Construction: wrap mylar in the shape of a cylinder around untreated potted rice plants and cover them with gauze (Fig. 1) secured with twine, rubber band, or elastic fabric. Or use a fabric bag instead of mylar.

e) Collect egg masses after 5-6 days by clipping and trimming leaves into pieces 2-4 cm long. Egg masses can be surface sterilized by soaking in 10% (v/v) formalin for 10 minutes and rinsed with slow-running tepid water for 15 minutes.

f) Two options for egg hatch:

1) Place leaf pieces with egg masses in petri dishes (14-cm diameter) with damp filter paper (Fig. 2A), covered with a lid. Ensure there is sufficient air flow.

2) Place egg masses in glass vials (Fig. 2B). Add cut pieces of rice leaflets to serve as food for emerging neonates. Do not let condensation accumulate inside vials (emerging neonate larvae can drown in water droplets). Add absorbent paper or cotton to reduce humidity as needed.

g) Four options to rear larvae to L2 or L3:

1) Rice Seedlings. Germinate rice seeds in a cup for 6 days (Fig. 3A). Ensure the seedlings are provided with enough moisture. Transfer neonates to the seedlings. Change the old rice with fresh rice seedlings every 2-4 days. Check development. After about 10 days, collect L2 for testing.

2) Potted Plants. Transplant rice seedlings into pots and grow for 50 days, or until desired height is reached. Transfer neonates to the potted plants (Fig. 3B) and place them in cages to protect against predation. Larvae transfer on their own from dead tillers to new ones. At 10-14 days after infestation, collect L2 larvae from tillers that dry up due to dead heart.

3) Cut Tillers. Transplant rice seedlings and grow for 50 days. Harvest and clean the largest tillers. Remove excess leaves and roots. In a water-proof container, add 1-2 cm of water, line the bottom with metal screen, and insert the tillers into the screen with the trimmed roots submerged (Fig. 3C). Add neonates by placing them near the base of tillers, and let them crawl onto the tillers. Add fresh tillers weekly as the old ones dry up. After 10-15 days, open the infested tillers, and collect L2 larvae.

4) Cut Stems. Transplant rice seedlings and grow for 50 days. Harvest and clean the largest tillers. Remove excess leaves and roots. Cut stems into pieces (4-5 cm), and add them to cells of a rearing tray, 3-5 per cell (Fig. 3D). Add neonates, one per stem. After 10-14 days, collect L2 larvae from stems.

Treatment

h) Prepare dilutions of insecticide in glass beakers or disposable plastic containers according to a dose range (e.g., 20, 5, 1.25, 0.31, 0.078, 0.0195 ppm) that includes 5-8 concentrations and a solvent control that provide a full range of mortalities (see Validity Criteria). Include a non-ionic surfactant e.g. 0.05% (v/v) Triton X-100 for better coverage.

i) Treatment of plants can be done one of two ways. Include at least observations for each treatment.

1) Plant Dip (Fig. 4A, B, C). Prepare 4-week old rice plants (25 cm). Dip rice plants in each treatment solution for 10 seconds, and dry in air for 2 hours. Cut rice stems at the base and remove foliage, leaving a 5 cm stem. In Petri dishes (9-cm diameter), add 3 filter papers and 3 mL of water to each, and maintain humidity during the study. Add 20 stems to each dish. Add ten L2 or L3 larvae per dish, with use of paintbrush or forceps. Seal the dish with a cotton filter. Each dish represents a separate observation.

2) Stem Dip (Fig. 4D, E, F). Prepare 4 to 6-week old rice plants. Cut stems to 4-5 cm, leaving roots attached. Wash to remove soil. Dip stems in each treatment solution for 10 seconds. Allow stems to dry for 2 hours. Prepare trays by adding a piece of moistened fiber or agar to each cell to maintain humidity. Add one piece of stem per cell and infest each with one L2 or L3 larva. Cover cells with lids. One observation is represented by 6-8 cells.

j) The Stem Dip method is preferred for *Scirpophaga incertulas*, due to need for larger plants.

Incubation

k) Store the assay containers at 25°C and 60% RH with a 16L:8D photoperiod with indirect light.

l) Assess mortality between 3-7 days after treatment (test length may vary depending on the AIs being evaluated). Mortality can also be recorded daily to determine changes in mortality over time. For longer experiments, provide a water source in each dish. Insects unable to right themselves when probed should be considered dead.

m) Express results as a percentage mortality. Correct for 'untreated' (control) mortalities using Abbott's formula[1] (Abbott 1925). The corrected mortality data can be subjected to a probit or logit dose response analysis to calculate an LC_{50} or LC_{90}.

[2] Corrected % mortality = (% alive control - % alive treated) x 100% / (% alive control)

[1] Abbott's formula: $Corrected\% = \left(1 - \frac{nT}{nC}\right) * 100$

nT = survivors in treatment.
nC = survivors in control.

n) If mortality is greater than 20% for the solvent control treatment, the study should be considered as invalid. Mortality at the highest rate must be 100%, and at least three datapoints should have mortality >0% and <100%.

o) For more information on validation, refer to "IRAC Susceptibility Test Methods Series." https://irac-online.org/test-methods/

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:

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.
2. Püntener W., 1981 Manual for field trials in plant protection second edition. Agricultural Division, Ciba-Geigy Limited.
3. Gao, C., Yao, R., Zhang, Z., Wu, M., Zhang, S., and Su, J. (2013). Susceptibility baseline and chlorantraniliprole resistance monitoring in *Chilo suppressalis* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 106: 2190-2194.
4. IRAC Susceptibility Test Methods Series. Insecticide Resistance Action Committee, Test Methods. URL: https://irac-online.org/teams/methods/documents (accessed 1/12/23).
5. Methodology was provided by Syngenta and FMC.

Fig. 1. Mylar cages for oviposition by adult moths on potted rice plants

A 

B 

Photographs provided by Syngenta (A) and FMC (B)

Fig. 2. Hatching eggs in a Petri dish or in glass vials

A 

B 

Photographs provided by Syngenta (A) and FMC (B)

Fig. 3. Methods for rearing neonates to second instar using rice (A) seedlings, (B) potted plants, (C) cut tillers, and (D) cut stems.

A 

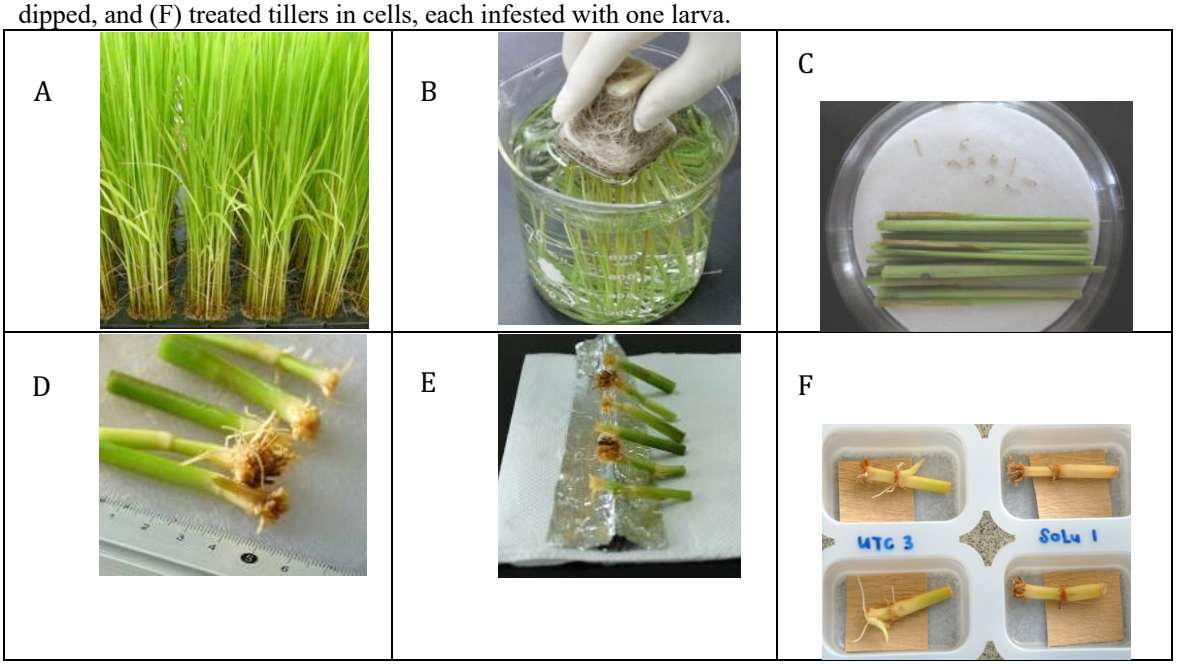
B 

C 

D 

Photographs provided by Syngenta (A) and FMC (B-D)

Fig. 4. (A) Four-week old rice plants used for dipping, (B) dipping for 10 seconds, (C) and a Ø 9-cm Petri dish with 20 stems infested with 10 larvae; (D) rice tillers used for dipping, (E) tillers drying after being dipped, and (F) treated tillers in cells, each infested with one larva.



Photographs provided by Syngenta (A-C) and FMC (D-F)

Fig. 5. Example data collection sheet.

Population	Treatment	Compound	Rate (ppm)	Dead				Alive				Dead Total	n	% Control	% Corrected Mortality
				Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4				
	1.1	CTPR Prevathon	20												
	1.2		5												
	1.3	50SC	1.25												
	1.4		0.31												
	1.5		0.078												
	1.6		0.0195												
	Control		0												