IRAC BIOTECH Susceptibility Test Methods Series

Method No: 1

Version: 3.2

Details:

Method:	No: 1	 Ostrinia nubilalis (Hubner) *Photograph Courtesy of: Frank Peairs, Colorado State University, United States*
Status:	Approved	
Species:	Maize borers (*Ostrinia spp., Sesemia spp., Diatraea spp.*) and other maize feeding Lepidoptera amenable to rearing on artificial diets	
Species Stage	Neonate Larvae	
Product Class:	Maize hybrids expressing Cry protein (Bt) traits such as Cry1Fa, Cry1Ab	
Comments: None		

Description:

Materials:

1. 128 well trays, each well 16 mm diam. x 16 mm height and vented lids to cover (CD International, Pitman, NJ)
2. Purified Cry protein of known percent active
3. Buffer solution: 0.1%Triton-X 100
4. Artificial diet suitable for the corn borer species
5. Disposable pipettes suitable for piping measured aliquots of diet into wells and diluted protein onto the cooled diet surface
6. Laboratory scale and disposable weigh boats suitable for weighing small aliquots of protein and small larvae
7. Growth chamber where temperature and light is controlled and recorded
8. Laboratory hoods that reduce contamination

Method: (Marçon et al. 1999)

1. A population sample that targets sufficient individuals to represent 200 genomes.
    a. A known susceptible laboratory colony
    b. Field collected populations consist of either adults obtained from light traps or from sweep net samples; or eggs or larvae collected from non-Bt maize
2. Collected individuals are reared on artificial diet in the laboratory to obtain eggs that will hatch into sufficient neonates to conduct the replicated assays (use rearing methods appropriate for species)
    a. Bioassay of neonate larvae involves exposure to Bt protein solutions applied to the surface of single wells of artificial diet.
    b. Preference is to utilize progeny (F_1) obtained directly from field-collected insects whenever possible. If required, insects from later generations (F_2 or F_3) can be used, or in cases where sufficient eggs are collected from the field, the hatching

neonates (F_0) can be assayed directly.

3. Neonates <24 hour old are placed in individual wells with 1 ml of diet onto which a measured concentration of protein has been applied uniformly to the diet surface
4. Sixteen neonates tested per protein concentration
5. A range of ~ 8 protein concentrations are selected using known susceptible insects, that span from no protein with 0% mortality (control) to a concentration that causes 100% mortality
6. Three replications per concentration (48 neonates) are tested for a total of ~384 neonates tested per population
7. After 7 days, mortality and individual larval weights are recorded for each well.
    a. Larva that have not grown beyond 1st instar and weigh ≤0.1 mg are considered dead
    b. Surviving larvae can be weighed as a group by replicate and an average weight calculated for each replicate
8. Statistical analysis of mortality is conducted using probit analysis
9. Larval weights are transformed to percentage growth inhibition relative to controls and the resulting data analyzed by nonlinear regression fitted to a probit model
10. LC and EC values are used to determine relative sensitivity of the tested populations
    a. EC_{50} calculations have been the most sensitive and most economical for detecting changes in susceptibility; however, an approximation of the upper limit of the? LC_{99} is most frequently adopted as a discriminating dose for confirming possible resistance in high dose pests. >1% survival at this concentration is considered statistically significant.

Percautions & Notes:

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1. Disposable plastic equipment is necessary to avoid contamination.
2. Laboratory sanitation and other operations that reduce potential contamination of diet and protein solutions is critical to consistent results.

Larvae that are prone to tunneling as neonates might not be suited to this diet surface dosing method

References & Acknowledgements:

Marçon, P.R.G.C., L.J. Young, K. Steffey, and B.D. Siegfried. 1999. Baseline susceptibility of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) to *Bacillus thuringiensis* proteins. J. Econ. Entomol. 92: 279-285.