Adult Vial Test (AVT) Protocol for:
Evaluation of the Susceptibility of *Helicoverpa zea* to Pyrethroid Insecticides

Order/Family: Lepidoptera: Noctuidae

Common names: cotton bollworm, corn earworm, tomato fruitworm, soybean podworm, sorghum headworm

Title: Testing Susceptibility of *Helicoverpa zea* to Pyrethroid Insecticides
Primary Purpose: To assess the extent of pyrethroid resistance in *Helicoverpa zea*
Locations: North America (Mexico, Eastern U.S. and Southern Ontario, Canada)
Test Species: *Helicoverpa zea*
Life Stage: Adult
Insecticides: Cypermethrin

Sampling Methodology:

Pheromone Trap: Wire cone Hartstack traps (Harstack et al. 1979) or equivalent should be utilized. Hartstack Lepidoptera moth traps can be obtained from:

Southern U.S.: Davis Tool and Die; 226 CR 235, Abbeville, MS, 38601 (601-234-4007)
Northern U.S.: Bob Poppe's Service; 25738 N. 3200 Rd., Lexington, IL, 61753 (309-723-3201)

Pheromone Blend and Source: The traps should be baited with *Helicoverpa zea* pheromone lures (Hendricks et al. 1987) to collect male moths throughout the sampling season. In general, the sampling season will extend from June through August/early September. Each lure should only be used for a period not to exceed 2-3 weeks.

One Source: Always use the Hercon "Corn Earworm/Cotton Bollworm Lure Tape" Lures:
Great Lakes IPM; Web: http://www.greatlakesipm.com/ (Catalog #: 700310, $16.00/pack (10))
10220 CHURCH ROAD
VESTABURG, MI 48891-9746
TEL: (989) 268-5693 / (989) 268-5911 TOLL FREE: 1-800-235-0285

Trap Placement: Place traps in open areas near the edge of cotton/corn fields. It may be necessary to move traps to optimize moth collection (e.g., to fresh-silking corn fields). Traps should be placed upwind of the likely source of moths. Moths may be obtained by light trapping and "sugar line" trapping also.

1)--ADULT VIAL TEST METHODOLOGY (Conventional SINGLE DOSE Method):

Moth Collection: Male moths will be collected from the traps in the morning, when moths are less active. Only fresh/healthy moths should be used in the assays. The collected moths will be maintained overnight (ca. 24 hrs) and fed a 10% sucrose solution. The wings of the moths will be examined and a general assessment of health will be made. The wings of healthy moths will have scales over almost the entire surfaces of the wings. Moths whose wings have lost most of the scales, or whose wings are damaged, should not be used for the adult vial test.

Place one moth in each vial. Screw the vial cap onto the vial completely and then turn the cap back 1/4 turn. Place the vials back into the shipping flats and hold the vials on an angle (tilted) for 24 hours at room temperature (ca. 24° C). Make sure that the room temperature is recorded on the data sheet.

Mortality counts will be taken 24 h after the test has been initiated. The moths will be evaluated as either alive, dead, or knocked-down. Knocked-down moths are those moths that are alive but unable to fly in a normal manner. Uncap the treatment vial and turn the uncapped vial "upside down". Moths able to fly ≥ 3 meters will be considered alive. Moths not able to fly > 3 meters will be recorded as "knocked down". "Knocked down" moths will be evaluated further by tossing them into the air. All data will be corrected for control mortality using Abbott's (1925) formula. Treated vials will be used once only.

Obviously, test dates and numbers of moths tested will depend upon trap catches. Ideally, 25-100 moths should be tested at each rate for a given sample, from June, mid-July or August/early September. Therefore, each rate (i.e., **10 μg cypermethrin/vial**) should have a minimum of 100-300 moths evaluated, depending on results. For example, if after 100 moths, results are indicating nearly 100% survival or 100% mortality, a smaller sample size can be used.

If survival of the diagnostic rate(s) occurs (based on the results obtained from the vial assay), it will be necessary to repeat the test at the same location with a new set of treated vials (from another lot if possible). If the moths are surviving in the vials treated with 10 μg cypermethrin/vial, vials treated with a higher dosage (30, 100 and 300 μg cypermethrin/vial may be obtained by contacting Dr. Greg Payne (Department of Biology, State University of West Georgia, Carrollton Georgia 30118, Tel. 678-839-4040, E-Mail: gpayne@westga.edu). It may be useful to collect larvae or eggs from those sites in order to establish a colony for more detailed tests (contact Greg Payne or Bill Hutchison or Eric Burkness for details; see below; page 3).

2)--ADULT VIAL TEST METHODOLOGY (MULTI-DOSE Method):

In addition to the single-dose assays (e.g., 5 or 10 ug of cypermethrin/vial), some cooperators may also be using the Multi-dose assay, where at least 5 or 6 total concentrations are used (e.g., 0.5 to 30 or 60 ug/vial). The primary goal of this assay is to estimate an LC-50 for a given location, but also compare data at 5 and 10 ug/vial, with other locations.

ALL Methods mentioned above will be used for this protocol as well. The primary difference is the sample size required per dose. Due to statistical requirements of a multi-dose approach, we may be able to reduce the total sample size for a given location.

SAMPLE SIZE: For each collection date, wait until you have at least 75 to 125 moths to work with. After collection, for a given collection date, use a minimum of 15 to 20 moths per dose, including moths needed for the acetone-treated check vials (again, one moth/vial). The first collection will be considered Replication #1; as the moth flight continues, repeat this for additional days (replications), at least 2 to 3 times (3-4 reps total). The data will be pooled for final regression analysis (fitting to a probit model; estimation of LC50).

DATA RECORDING and REPORTING:

Data should be recorded for each assay on a data record sheet. DATA sheets will be sent separately. One copy should be kept by the cooperating researcher, and one copy should be sent to each of the following coordinators:

DATA REPORTING CONTACTS:

For the Southern Region:

Dr. Greg Payne,
Dept. of Biology
State University of West Georgia,
Carrollton, GA 30118,
Tel. 678-839-4040
E-Mail: gpayne@westga.edu

Midwest Region:

Mr. Eric Burkness
Dept. of Entomology
University of Minnesota
St. Paul, MN 55108
Ph: 612-624-3670
Fx: 612-625-5299 (att: Burkness)
Email: burkn001@umn.edu

FMC Cooperators (Midwest Region):

Mr. Len Dobbins
FMC Corporation
Commercial Business Specialist
19866 Moontown Road
Noblesville, IN 46060
317-409-1990 (cell)
317-867-5999 (Office)
317-867-5998 (Fax)
Email: len_dobbins@fmc.com

Use of Data:
The adult vial tests are conducted using diagnostic rates to detect early shifts in susceptibilities and to identify areas where further testing is needed. These evaluations do not necessarily indicate field resistance. Results should not be released to the public or published in newsletters prematurely. A lead author will summarize the results for the Beltwide Cotton Conferences, Cotton Pest Management Conferences, and for meetings of the Midwest Food Processors Assoc., State Vegetable Grower Associations in the Northern U.S., listing all cooperators as co-authors. A written final report will be submitted to all funding agencies and for publication in the Proceedings of the Beltwide Cotton Conferences, Midwest Food Processor Association, and other relevant scientific and grower-based meetings. After these reports have been completed, all cooperators are free to use their individual data or access the full set of data for any individual uses.

NOTE:
To provide a more rapid data entry and reporting system, a web-form is being developed, beginning with the 2006 field season. Once this is available, you will be given a password, username, and instructions for data entry. However, in the meantime, and throughout the course of the project, please continue to maintain your hard-copy records of data collected at your location(s).