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SURVEY OF ACETYLCHOLINESTERASE ACTIVITIES IN FIELD-COLLECTED TOBACCO BUDWORM AND BOLLWORM POPULATIONS FROM GEORGIA-2004

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Abstract

Adult-head acetylcholinesterase (AChE) activity from a methyl parathion-resistant strain (OPR) of tobacco budworm, *Heliothis virescens* (F.), was 21-fold less sensitive to methyl paraoxon when compared to AChE activity from a methyl parathion-susceptible strain (OPS). Acetylcholinesterase activities from Georgia field strains of tobacco budworm and bollworm (*Helicoverpa zea* (Boddie)), were determined also. Acetylcholinesterase activities from the heads of OPR moths were less sensitive to inhibition by propoxur, an N-methyl carbamate insecticide, and inhibited by the organophosphate insecticide, monocrotophos; whereas, OPS moth AChE was less sensitive to monocrotophos and inhibited by propoxur. Between 1995-1997, acetylcholinesterase activities for field strains of tobacco budworm were comparable to the OPS strain or intermediate. Acetylcholinesterase activities from field colonies collected during 2003-2004 were intermediate or more comparable to the OPR strain. Despite significant decreases K_i values over the ten-year study period, LD_{50}s have remained constant or only increased slightly (1.2-fold). These data suggest that resistance to organophosphate insecticides in the more recent field strains may be due to increased expression of an altered acetylcholinesterase gene within these populations.

Introduction

Resistance to insecticides has been documented in more than 500 species of arthropods and is often associated with an increased enzymatic detoxification of an insecticide or reduced sensitivity of a target protein to inhibition or binding by the insecticide (Soderlund and Bloomquist 1989; Brown 1990). Decreased sensitivity of acetylcholinesterase (AChE) to inhibition by organophosphate and carbamate insecticides has been implicated as a mechanism of resistance to those chemicals in a variety of insect species (Hama 1983; Gilbert et al. 1996). In order to detect and monitor the development of resistance in field populations of pest insects, a number of biochemical and genetic methods have been developed and implemented Brown and Brogdon 1987). One such method that has been adopted is the use of a "microtiter plate assay" to estimate the activity of AChE in response to inhibition by organophosphate and carbamate insecticides in tissue preparations from single insects (Brogdon 1988; Moores et al. 1988).

Herein, we report the results from a survey of AChE activities of adult heads from laboratory-maintained and field-collected strains of tobacco budworm (*Heliothis virescens*; TBW) and bollworm (*Helicoverpa zea*) using a microtiter plate bioassay. The 1995-1997 data have been previously reported (Thompson et al. 1998), but have been included in this presentation for comparison to more recent data collected during the 2003-2004 seasons.

Research Methods

TBW and bollworm larvae and adults were collected from various host crops (i.e, cotton, corn, tobacco, and chickpea) throughout the state of Georgia and transferred to facilities at the State University of West Georgia. The following strains were used:

Tobacco Budworm Strains

OPS—laboratory-maintained organophosphate-susceptible strain obtained from T. Brown (Clemson University)
OPR—laboratory maintained organophosphate-resistant strain obtained from T. Brown (Clemson University)
Dec-95—collected as larvae from non-Bt cotton; Decatur County, GA
Tif-95—collected as larvae from non-Bt cotton; Tift County, GA
EarA-96—collected as larvae from non-Bt cotton; Early County, GA
EarB-96—collected as adults from non-Bt cotton; Early County, GA
Mil-96—collected as adults from a non-Bt cotton border plot; Miller County, GA
Tif-96—collected as larvae from non-Bt cotton; Tift County, GA

Bak-97—collected as adults from non-Bt cotton; Baker County, GA
Col-97—collected as larvae from tobacco; Colquit County, GA
Dec-97—collected as larvae from non-Bt cotton; Decatur County, GA
Ear-97—collected as larvae from non-Bt cotton; Early County, GA
Mil-97—collected as larvae from non-Bt cotton; Miller County, GA
MitA-97—collected as adults from non-Bt cotton; Mitchell County, GA
MitB-97—collected as larvae from non-Bt cotton; Mitchell County, GA
Tif-97—collected as larvae from tobacco; Tift County, GA
Tif-03—collected as larvae from tobacco; Tift County, GA
Bur-04—collected as adults from Bt cotton; Burke County, GA
Doo-04—collected as larvae and adults from Bt cotton; Dooly County, GA
Ter-04—collected as larvae and adults from Bt cotton; Terrell County, GA

Bollworm Strains

EarA-96—collected as larvae from non-Bt cotton; Early County, GA
EarB-96—collected as adults from non-Bt cotton; Early County, GA
Mit-96—collected as adults from a non-Bt cotton; Mitchell County, GA
Dec-97—collected as larvae from non-Bt cotton; Decatur County, GA
Ear-97—collected as larvae from non-Bt cotton; Early County, GA
Mil-97—collected as larvae from non-Bt cotton; Miller County, GA
Mit-97—collected as adults from non-Bt cotton; Mitchell County, GA
Ear-04—collected as larvae from corn; Early County, GA
Mac-04—collected as larvae from corn; Macon County, GA
Pea-04—collected as larvae from Bt cotton; Peach County, GA
Ter-04—collected as larvae and adults from Bt cotton; Terrell County, GA

Larvae were transferred to a pinto bean/wheat germ, agar-based diet, and adults were placed in mating cages to produce adequate numbers of larvae and adults for testing. Larvae and adults were maintained at 27°C, LD 14:10 and ca. 40% RH.

Acetylcholinesterase activities were determined according to the methods of Ellman et al. (1961) and Brown and Bryson (1992). Individual heads were homogenized in 0.5 ml MOPS buffer (0.1M, pH 7.5) using 10 strokes of a hand-held glass tissue homogenizer (Kontes, Model 20). Each homogenate was transferred to a 1.5 ml microcentrifuge tube and centrifuged for 5 min in a Beckman bench top microcenrifuge. Supernatants were transferred to clean 1.5 ml microcentrifuge tubes and used as the enzyme source. Reaction mixtures (with and without inhibitor) were prepared as follows: the inhibitor (in 100 µl of acetone) or 100 µl of acetone alone was mixed into 15 ml of MOPS buffer containing 0.6 mM acetylthiocholine iodide and 2.4 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). To each well of a 96-well microtiter plate, 20 µl of enzyme were added. Reactions were initiated by the addition of 100 µl of an appropriate reaction mixture to the enzyme. AChE activiites (mOD/min) were determined using a microtiter plate reader by measuring absorbance (405 nm) throughout a 40 min time period. Bimolecular reaction constants were obtained by plotting the percent activity remaining against time and the slope of the regression was divided by the inhibitor concentration (Hart and O'Brien 1973). Susceptibility tests were performed by topical application of a 1 µl aliquot of a test solution (or acetone) to the dorsal thorax of fourth instar larvae. Treated larvae were held at 27°C and 60% RH. Mortality was assessed at 48 h post-treatment, and probit analyses (SAS) were used to determine the median lethal dose (LD50) for each treatment. Results were adjusted for the average weight of larvae (ca. 35 mg) in each test and control mortality (Abbott's Formula).

Results

Bimolecular rate constants of AChE from each of the TBW (Table 1, Figure 1) and Bollworm (Table 2, Figure 2) strains were determined. Adult, head AChE activity from the OPR strain was 21-fold less sensitive to inhibition by methyl paraoxon when compared to AChE activity from the OPS strain. AChE activity of the OPS strain was most rapidly inhibited by methyl paraoxon; whereas the AChE activity of the OPR strain was least inhibited. During 1995-1997, bimolecular reaction constants for the TBW field strains were comparable to that obtained for the OPS strain or intermediate (Ki values between 18-45 M-1min-1). During the 2003-2004 season, the bimolecular reaction constants for several strains (i.e., Tif-03,

Bur-04 and Doo-04) were greatly reduced indicating a decreased AChE sensitivity to inhibition by methyl paraoxon. Bimolecular rate constants for Bollworm AChE have decreased throughout the study period also. During 1996-1997, values ranged between 31-48 M-1min-1; in 2004, values between 16-18 M-1min-1 (an ca. 2-fold decrease) were recorded.

Table 1. Bimolecular Reaction Constants for Acetylcholinesterase from Tobacco Budworm Adults Following Exposure to Methyl Paraoxon.[a]

Strain	K_i x 10^{-3} (SE)[b]; Ratio[c]
OPS	40 (4.3)
OPR	1.9 (0.7); 21
Dec-95	23 (0.7); 1.7
Til-95	21 (1.9); 1.9
EarA-96	20 (2.8); 2.0
EarB-96	18 (3.7); 2.2
Mil-96	26 (1.9); 1.5
Til-96	43 (4.5); 0.9
Bak-97	45 (2.3); 0.9
Col-97	34 (3.1); 1.2
Dec-97	39 (1.6); 1.0
Ear-97	22 (2.6); 1.8
Mil-97	35 (3.4); 1.1
Mit-97	24 (2.8); 1.7
Til-97	33 (4.3); 1.2
Til-03	6.5 (0.7); 6.2
Bur-04	8.5 (1.1); 4.7
Doo-04	7.2 (0.6); 5.5
TerA-04	25 (1.9); 1.6
UWG-04	22 (3.3); 1.8

[a] Pooled activities from individual heads; inhibited in the presence of methyl parathion
[b] Bimolecular reaction constants ($M^{-1}min^{-1}$)
[c] Quotient of Ki OPS divided by Ki

Figure 1. Inhibition Constants (K_i) for Acetylcholinesterase from Tobacco Budworm Adults Following Exposure to Methyl Parathion[a]

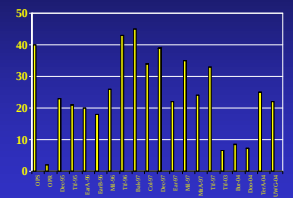
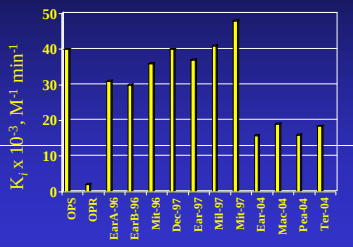


Table 2. Bimolecular Reaction Constants for Acetylcholinesterase from Bollworm Adults Following Exposure to Methyl Paraoxon.[a]

Strain	K_i x 10^{-3} (SE)[b]; Ratio[c]
OPS	40 (4.3)
OPR	1.9 (0.7); 21
EarA-96	31 (2.8); 1.3
EarB-96	30 (1.9); 1.3
Mit-96	36 (3.6); 1.1
Dec-97	40 (2.3); 1.0
Ear-97	37 (1.7); 1.1
Mil-97	41 (2.6); 1.0
Mit-97	48 (3.2); 0.8
Ear-04	16 (0.8); 2.5
Mac-04	18 (2.8); 2.2
Pea-04	16 (0.9); 2.5
Ter-04	18 (1.8); 2.2

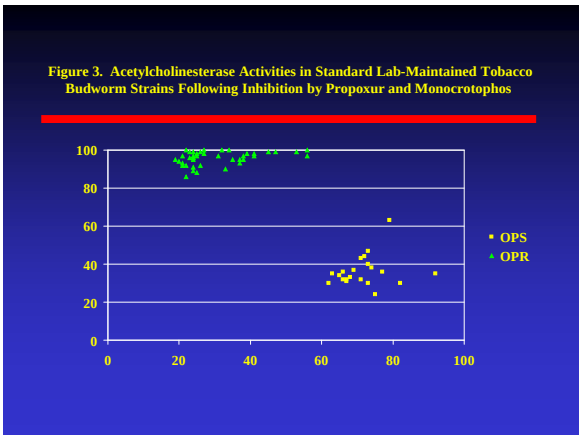
[a] Pooled activities from individual heads; inhibited in the presence of methyl parathion
[b] Bimolecular reaction constants ($M^{-1}min^{-1}$)
[c] Quotient of Ki OPS divided by Ki

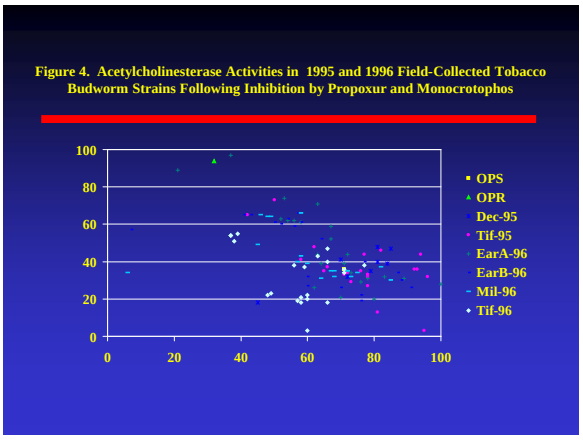
Figure 2. Inhibition Constants (K_i) for Acetylcholinesterase from Bollworm Adults Following Exposure to Methyl Parathion[a]

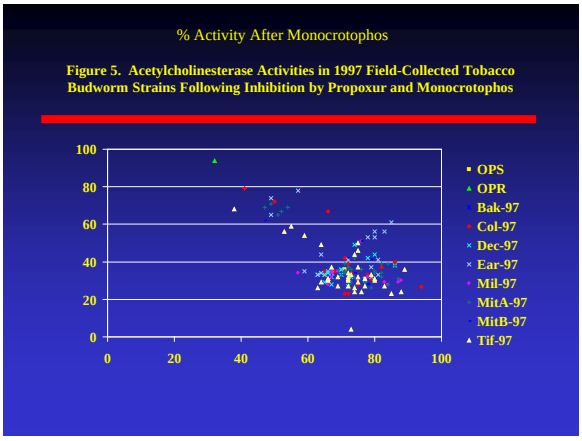


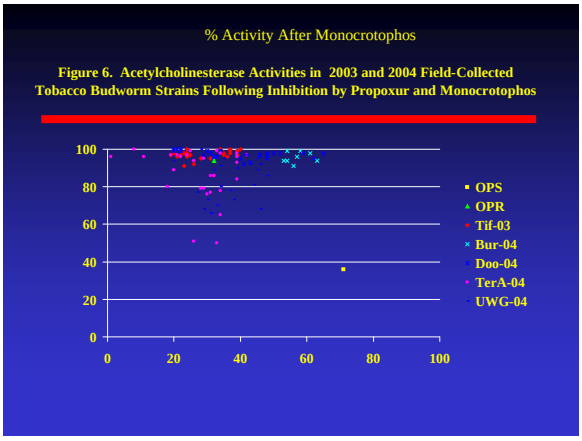
Acetylcholinesterase activities were assessed from each of the strains following inhibition by propoxur (an N-methyl carbamate and selective inhibitor of OPS AChE activity) and monocrotophos (an organophosphate and selective inhibitor of OPR AChE activity (TBW: Figures 3-6; Bollworm: Figure 7). All of the OPS responses were in the lower right quadrant of the scatterplot; whereas, all of the OPR responses were in the upper left quadrant of the scatterplot (Figure 3). The responses of most of the 1995-1997 TBW field-collected strains to inhibition by propoxur and monocrotophos were similar to the OPS response or intermediate (Figure 4, Figure 5). Responses of the 2003-2004 TBW strains were intermediate or more closely resembled the OPR response (Figure 6). Although primarily clustered in the lower right quadrant, Bollworm AChE activities were more varied (Figure 7).

Of the few field-collected strains of TBW evaluated, all demonstrated a decreased sensitivity or resistance to methyl parathion (Table 3). LD_{50} values for the field-collected TBW strains ranged from 126-192 µg/g larva, a 15-22-fold level of resistance as compared to the OPS strain.









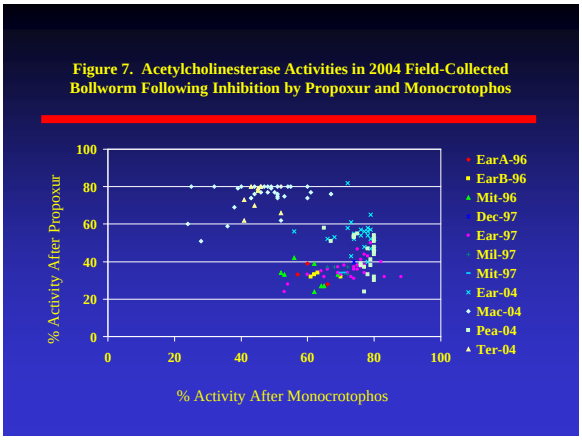


Table 3. Susceptibility of Tobacco Budworm Larvae to Methyl Parathion[a]

Strain	LD_{50} µg/g larva (Slope)	RR[b]
OPS	8.6 (1.9)	-----
OPR	317 (0.9)	36.9
Dec 95	126 (1.9)	14.6
Tif 95	151 (2.5)	17.6
Doo 04	135 (1.4)	15.7
Ter 04	189 (1.5)	22.0
UWG 04	192 (3.2)	22.3

[a] topical application
[b] LD_{50}/OPS LD_{50}

Conclusions

The OPR AChE was 21-fold less sensitive to inhibition by methyl paraoxon as compared to OPS AChE activity. AChE activities for the 1995-1997 TBW stains and the Bollworm strains were intermediate or more comparable to OPS; however, AChEs isolated from several of the more recent TBW strains were less sensitive to inhibition. Since genetic linkage analyses have indicated that AChE inhibition characteristics were controlled by a single gene, *Aceln*, and that the SS genotypes were fully susceptible to propoxur, the RR genotypes were fully susceptible to monocrotophos, and the RS genotypes were partially inhibited by either compound, our data suggested that the 1996-1997 TBW field populations were composed of SS and RS genotypes and the 2003-2004 TBW populations were more predominantly composed of RS and RR genotypes. Based on these data, the Bollworm populations evaluated would be presumed to be composed of the SS and RS genotypes also.

In general, trends in the susceptibility data support the enzyme inhibition data. Susceptibility data indicated that several of the field strains were resistant to methyl parathion, and although other resistance mechanisms (i.e., decreased penetration and increased detoxification) may be a factor (especially in the 1996-1997TBW populations), an altered AChE may contribute to resistance in some of the more recently collected strains.

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