ENZYME ACTIVITIES IN ORGANOPHOSPHORUS-RESISTANT TOBACCO BUDWORMS *HELIOTHIS VIRESCENS*

John A. Harold and James A. Ottea
Dept. of Entomology, LSU Agricultural Center
Baton Rouge, LA

Abstract

Frequencies of profenofos resistance and enzymes activities associated with metabolism of organophosphorus (OP) insecticide were measured in larvae of the tobacco budworm, *Heliothis virescens* (F.) that were field-collected from Northeast Louisiana during the 1995 and 1996 cotton growing seasons. High frequencies of profenofos resistance were recorded in all field-collected strains and expression of resistance was strongly correlated with esterase (EST) activity toward a-naphthyl acetate but not P450-monooxygenase (P450-MO) activity toward *p*-nitroanisole. Moderate correlations were measured between profenofos resistance and glutathione S-transferases (GST) activity toward 1-chloro-2,4-dinitrobenzene (CDNB), but not 1,2-dichloro-4-nitrobenzene; DCNB. Finally, there was no association between profenofos resistance in all field strains and sensitivity of acetylcholinesterase (AChE) to inhibition by chlorpyrifos oxon, although in some strains, mean levels of sensitivity were reduced relative to that of an insecticide-susceptible strain. These results suggest that rapid assays measuring EST activity toward a-naphthyl acetate may be useful for monitoring metabolic resistance to OP insecticides in tobacco budworms.

Introduction

The tobacco budworm, *Heliothis virescens* (F.) causes significant economic damage to a wide range of field and horticultural crops (Sparks, 1981). During the 1995 growing season, *H. virescens* together with the corn earworm, *Helicoverpa zea*, infested nearly 82% of all U. S. cotton causing 36% of all insect-related losses to this crop (Williams, 1996).

Insecticide resistance is a major factor contributing to our inability to manage populations of this pest (Sparks et al., 1993). Insecticide resistance may result from reduced cuticular penetration, increased detoxication, and/or altered sites of action of insecticides (Oppenoorth, 1985). A number of studies have shown that all three factors may contribute to OP resistance in tobacco budworms (Sparks et al., 1993). In an early study, activity of P450 monooxygenases (P450-MOs; EC 1.14.14.1) in midgut preparations of OP-resistant tobacco budworms was greater than that of susceptible insects (Reed, 1974). In a more recent study of methyl parathion resistant tobacco budworms, P450-MO activity responsible for desulfuration of methyl parathion was lower, and phosphorotriester hydrolase activity responsible for methyl paraoxon hydrolysis was higher, in resistant than susceptible insects (Konno et al., 1989). Whereas activities of glutathione *S*-transferases (GSTs; EC 2.5.1.18) were not different in these studies, the role of these enzymes in OP metabolism and resistance is well established (Oppenoorth, et al., 1979). Altered sensitivity of acetylcholinesterase (AChE: EC 3.1.1.7) to inhibition by OPs and carbamates has been measured in strains of the tobacco budworm and is also a suspected resistance factor (Kanga and Plapp, 1994;). Sensitivity of AChE to inhibition by methyl paraoxon was reduced by 19- to 22-fold in larvae and adults of a methyl parathion-resistant strain of *H. virescens* (Brown, 1990). In addition, results from recent studies suggest that insensitivity of AChE is a major mechanism of resistance to OPs and carbamates in *H. virescens* together with reduced cuticular penetration (Kanga and Plapp 1994; Brown et al., 1996; Wolfenbarger, 1996).

The objective of this study was to measure actvities of enzymes associated with resistance to OP insecticides in laboratory and field populations of *H. virescens*. Results from this study show that activities of esterases (but not P450-MO) were highly correlated with frequencies of profenofos resistance. In addition, high GST/CDNB activities also were found to be associated with profenofos resistance although correlations were dependent upon location sampled.

Materials and Methods

Chemicals

Technical grade profenofos (*O*-(4-bromo-2-chlorophenyl)-*O*-ethyl-*S*-propyl phosphorothioate: 89%; Ciba-Geigy, Greensboro, NC) and chlorpyrifos oxon (O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphate: 100%; DowElanco Inc., Indianapolis, IN) were used for these studies. Reduced glutathione (GSH), glucose-6-phosphate, glucose-6-phosphate dehydrogenase, bovine serum albumin (fraction 5), nicotinamide adenine dinucleotide phosphate (NADP), *p*-nitroanisole (PNA), acetylthiocholine iodide (ATChI), 5,5'-dithio-*bis*(2-nitrobenzoic acid) (DTNB), and a-naphthyl acetate (a-NA) were purchased from Sigma Chemical Company (St. Louis, MO). Phenylthiourea (PTU), 1,2-dichloro-4-nitrobenzene (DCNB), 1-chloro-2,4-dinitrobenzene (CDNB), Fast Blue B salt, trichloroacetic acid (TCA), and potassium chloride (KCl) were purchased from Aldrich Chemical Company (Milwaukee, WI). Glycerol (USP grade) was purchased from EM Science (Gibbstown, NJ).

Insects

Three insect collections were made from unsprayed plots at the Louisiana State University Agricultural Center's Northeast Research Station/Macon Ridge location (MRS; Winnsboro, LA). Eggs and neonates were collected from

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1013-1018 (1997)
National Cotton Council, Memphis TN

a domesticated stand of velvet leaf, *Abutilon theophrasti* Medicus, on June 13, 1995 (MRS Jun), and from mature cotton on August 23, 1995 (MRS Aug). Adults were collected from Dallas grass, *Paspalum dilatatum* Poir. on September 14, 1995 (MRS Sep). An additional collection of eggs and neonates was made at the Red River (RR) Research Station (Bossier City, LA) on August 23, 1995 (RR Aug) from a field treated with four applications of cypermethrin, and one application each of thiodicarb, acephate and sulprofos. Larvae were separated following head capsule slippage at the end of the fourth stadium and fifth stadium (day 1-180±20 mg) were selected for biological and biochemical assays. Adults were reared in 3.8 l cardboard cartons covered with cotton gauze as a substrate for oviposition and provided with sucrose (10% in water) as a carbohydrate source (Leonard *et al.*, 1988). Both larvae and adults were held at 27oC, 14:10 LD, and 70% relative humidity.

Results from biological and biochemical assays with field-collected strains were compared with those from two laboratory strains. The LSU-S strain is a reference susceptible strain that was established from field collections from cotton in 1977 (Leonard *et al.*, 1988) and has been reared in the laboratory without intentional exposure to insecticides. An OP-resistant strain (OP-R) was established from the RR Aug F3 strain following selection of larvae with profenofos.

Biological assay

Susceptibility of *H. virescens* to profenofos was assessed following topical application of 1 µl profenofos (in acetone) to the thoracic dorsum of larvae. Dose-mortality response of LSU-S larvae was measured with five doses of profenofos (10 larvae per dose) and replicated thrice. Treated larvae were held in 1 oz cups with diet and absence of coordinated movement of a treated larva within 30 seconds after being prodded with a pencil was considered as the criterion for mortality, which was recorded 72 hr after treatment. Data were corrected for control mortality (≤ 3%) using Abbott's formula (Abbott, 1925) and subjected to probit analysis (Finney, 1971) using a micro-computer based program (SAS Institute Inc. Cary, NC). Frequency of resistance to profenofos in field-collected strains was assessed 72 hr following topical application of 15.3 µg profenofos/larva.

Tissue preparation

Tissues from individual larvae were used to measure the activities of P450-MO (midgut), AChE (head), and GST or EST (fat body). Individual larvae were weighed, decapitated and dissected. Individual midguts were split longitudinally and rinsed with ice-cold buffer (0.1 *M* sodium phosphate, pH 7.7) to remove gut contents, and transferred to an all-glass homogenizer. Fat bodies from individual larvae were obtained by gentle scraping and aspiration from the rinsed hemocoel using a Pasteur pipette. Midguts and fat bodies were homogenized in volumes of 500 and 400 µl, respectively, in ice-cold 1.15% KCl (containing a few crystals of PTU) using 10 strokes of an all glass homogenizer. Individual heads were homogenized in 250 µl of 0.1 *M* sodium phosphate buffer (pH 8.0) containing 0.1% Triton X-100. Homogenates were centrifuged at 4oC for 10 min at 10,000*g* (midguts) or at 12,000*g* for 15 min (fat bodies and heads). Resulting supernatants were held in ice and used in enzyme assays within 30 min of preparation.

Enzymes assays

Activities of P450-MO toward PNA were measured using the method of Kinoshita *et al.*, (1966) with modifications (Hansen and Hodgson, 1971; Kirby *et al.*, 1994). Reaction mixtures in duplicate contained (final concentrations in 200 µl total volume): a NADPH generating system consisting of NADP (0.11 m*M*), glucose-6-phosphate (2.4 m*M*), and glucose-6-phosphate dehydrogenase (0.25 Units), 0.1 *M* sodium phosphate buffer (pH 7.7) and enzyme homogenate (111 µl containing 0.222 insect equivalent (0.085±0.017 mg protein). Reactions were started by the addition of substrate (2 µl PNA in ethanol; 0.35 m*M* final concentration) and were incubated for 40 min at 27oC. Reactions were stopped by the addition of 5 µl of TCA (2.5% final concentration), and reaction mixtures were mixed vigorously and centrifuged at 10,000*g* for 5 min at 4oC. Supernatants (190 µl) were discharged into individual wells of a microtiter plate (Costar, Cambridge, MA) that contained 10 µl of 5 *N* sodium hydroxide, and absorbance was measured at 405 nm using a Thermomax microplate reader (Molecular Devices, Palo Alto, CA). Results were corrected for nonenzymatic activity using incubations without substrate and generating system as the control, then converted to pmoles of *p*-nitrophenol formed min-1 using an experimentally derived "extinction coefficient" of 10.41 m*M*-1 200 µl-1 (Kirby *et al.*, 1994).

Activities of GST toward DCNB or CDNB were measured following the methods of Booth *et al.* (1960) or Jakoby (1978), respectively, as modified by Grant *et al* (1989) and Kirby *et al.* (1994). Reactions were measured in 96 well microtiter plates that were prewashed with 2.5% Tween 20 (v/v in water). Substrate solutions (0.75 m*M*) were prepared by mixing sodium phosphate buffer (0.1 *M*, pH 8.0) containing 15% glycerol with stock solutions of CDNB or DCNB (50 m*M* in DMSO). Reaction mixtures in individual wells consisted of 200 µl of either DCNB or CDNB substrate buffer (0.5 m*M* final concentration), 30 µl GSH (8 m*M* final concentration), and enzyme homogenate (70 µl containing 0.065±0.11 mg protein for DCNB or 5 µl containing 0.013±0.002 mg protein for CDNB). Reaction volumes were made up to 270 µl using sodium phosphate buffer (0.1 *M*, pH 8.0). Reactions were initiated by addition of 200 µl of either DCNB or CDNB substrate buffer, incubated at 30oC and the rate of change in optical density (OD) during the initial 10 min of reaction was measured at 340 nm. Resulting data were corrected for nonenzymatic activity using incubations without protein as the control

and converted to nmol conjugate formed min-1 mg protein-1 using experimentally derived "extinction coefficients" of 8.5 or 10.09 m*M* -1300 µl-1 for DCNB or CDNB, respectively (Grant *et al*., 1989, 1991).

Esterase activity towards a-NA was quantified using the assay of Gomori (1953) with modifications (van Asperen, 1961; Grant *et al.*, 1989; Ibrahim and Ottea, 1995). Substrate solution was prepared by adding 600 µl of a-NA (0.113 *M* dissolved in 50% acetone) to a solution of Fast Blue B salt (18 mg in 30 ml of 0.1 *M* phosphate buffer, pH 7.0), and then filtered using Whatman #3 filter paper. Reaction mixtures in individual wells of a microtiter plate contained 240 µl of substrate solution (2.12 m*M* final concentration), 10 µl of homogenate (containing approximately 0.011±0.003 mg protein; 0.025 tissue equivalents). Reaction mixtures were incubated at 30o C and the change in absorbance during the initial 10 min was measured at 595 nm. Data obtained were corrected for non-enzymatic activity using incubations without protein as a control. Changes in OD were converted to nmol/min using the experimentally derived "extinction coefficient" (3.825 m*M*-1 250 µl-1) for the a-naphthol-Fast Blue B conjugate at 595 nm.

Sensitivity of AChE to inhibition by chlorpyrifos oxon was measured from larval heads using the method Ellman *et al*., (1961) with modifications (Byrne and Devonshire, 1993; Ibrahim and Ottea, 1995). Homogenates from individual heads (30 µl containing 0.022±0.004 mg protein; 0.12 tissue equivalent) were incubated for 10 min at 30oC in wells of a microplate containing 69 µl of sodium phosphate buffer (0.1 *M*, pH 8.0) and 1 µl of 148.8 n*M* chlorpyrifos oxon (final concentration). The concentration of chlorpyrifos oxon used in these studies was the I80 for the LSU-S strain as determined in preliminary experiments (data not shown). Activities measured in the absence of chlorpyrifos oxon served as the control. In incubations with eserine sulfate, maximum inhibition of AChE activity (92.1%) was measured at 20 µ*M* and did not increase at higher concentrations suggesting that the contribution of general esterases measured in these experiments was approximately to 9% of activity (data not shown). Percentage inhibition was calculated as 1-(activity with inhibitor/activity without inhibitor) X 100.

Enzyme activities are presented relative to protein concentration, which was measured using the assay of Bradford (1976) with bovine serum albumin (BSA) (fraction V; concentrations corrected for impurities) as the standard. Data were subjected to analysis of variance followed by Tukey's multiple comparison test (*P*=0.05) using a microcomputer based program (SAS Institute Inc. Cary, NC).

Results

Larvae from all field strains showed lower levels of susceptibility to profenofos than those from the laboratory reference LSU-S strain (Fig. 1). In bioassays with larvae from field-collected strains, mortality measured following topical application of a diagnostic dose ranged from 6.67% (RR Aug P1) to 56.67% (MRS Sep F3). The OP-R strain, which was selected from RR Aug F3 insects, had intermediate frequencies of OP resistance (36.67%).

Resistance frequencies in all field-collected strains decreased in the absence of insecticide selection (Fig. 1). Loss of resistance was most dramatic in MRS Aug and Sep strains following two generations of laboratory rearing. Whereas resistance frequencies in F1 generations of these strains were similar, resistance in F3 generations from MRS Aug and Sep decreased 3.25- and 5.67-fold, respectively. Similarly, in RR Aug insects, mortality measured after treatment with the diagnostic dose increased 3-fold in two generations from 6.67% (RR Aug P1) to 20.0% (RR Aug F2). An additional 2-fold increase in mortality was measured between RR Aug F2 and F4 generations.

Larvae from all field-collected strains expressed significantly higher activities of P450-MO than LSU-S larvae (Table 1). The highest levels of activity were measured in MRS Aug F3, RR Aug P1 and MRS Jun F3 larvae, and were 10.4, 8.7 and 7.5 times greater than activity in LSU-S, respectively (Table 1). Activity measured in OP-R larvae was 7.2-fold greater than that of LSU-S insects, and was statistically similar to activities measured in all field-collected strains except MRS Aug F3 and RR Aug P1. For all strains, P450-MO activities were not correlated (r2=0.00) with profenofos susceptibility. Correlations between activity and mortality also were poor when strains from the two sites collection were analyzed seperately (r2=0.01 and 0.07 for MRS and RR collections, respectively).

Effects of laboratory rearing on levels of P450-MO activity varied in all field-collected strains (Table 1). For RR Aug insects, activity decreased significantly from P1 to F2 generations. A subsequent increase in activity between F2 and F4 generations was not significant. Similarly, for MRS Jun and Sep strains, activity decreased following laboratory rearing although decreases were not statistically significant. In contrast, P450-MO activity in MRS Aug larvae increased significantly between F1 and F3 generations.

Activity of GST toward CDNB also was significantly higher in larvae from all field-collected strains than that of LSU-S larvae (Table 1). Activity was greatest in MRS Sep F1 larvae (10.2-fold greater than that of LSU-S) and did not differ significantly from RR Aug P1, MRS Aug F1, or MRS Jun F3. Activity of GST/CDNB measured from the OP-R strain was 7.3-fold greater than that of LSU, and was

intermediate relative to strains from field collections. Activity of GST/CDNB from all strains was moderately correlated with susceptibility to profenofos (r2=0.55), however, this correlation was higher in MRS (r2=0.75) than RR (r2=0.23) strains .

Levels of GST activity with CDNB measured in all field-collected strains were variable following laboratory rearing (Table 1). Significant decreases in activity were observed between MRS Jun F3 and F5, as well as between MRS Sep F1 and F3 strains. Activity also decreased between F1 and F3 generations of the MRS Aug strain, but this difference was not statistically significant. For RR Aug strains, activity of GST/CDNB decreased significantly in the first two generations (i.e., from P1 to F2) but was similar between F2 and F4 generation.

Insects from field collections expressed higher levels of GST activity towards DCNB than the reference LSU-S strain, but differences were not as great as those measured with CDNB (Table 1). Highest activities were measured in RR Aug P1 and MRS Sep F1, and were 5.5- and 5.1-fold higher than those from LSU-S, respectively. Levels of GST/DCNB activities in these strains were on par with all other strains except those of MRS Jun F3 and MRS Sep F3. Mean levels of activity in OP-R larvae were not different from those of field-collected strains, and were 4.0-fold higher than those of LSU-S. Correlations between activities of GST/DCNB and profenofos susceptibility were poor in all strains (r2=0.25) and analyses with individual sites of collections (r2=0.16 for MRS and 0.33 for RR strains, respectively). In contrast to results with CDNB, activity toward DCNB in strains of field collections did not change significantly after laboratory rearing (Table 1).

Esterase activities toward a-NA in larvae from all field-collected strains were significantly higher than those measured in LSU-S larvae (Table 1). Highest levels of esterase activity were measured in RR Aug P1 and F2 strains and were 3.3- and 2.9-fold higher than those of LSU-S larvae. Elevated levels of esterase activity also were measured in OP-R larvae, which were comparable to those measured in all field-collected strains. Esterase activities and profenofos susceptiblity were strongly correlated in analyses with all strains (r2=0.96), and individual sites of collections (r2=0.98 and 0.99 for MRS and RR Aug strain, respectively). Although variable levels of esterase activity were measured in larvae from field collections after laboratory rearing, these differences were statistically insignificant (Table 1).

Mean levels of AChE inhibition by chlorpyrifos oxon in larvae from field-collected strains were not significantly different from LSU-S except for RR Aug P1 (Table 1). In tests with RR Aug P1 larvae, low levels of AChE inhibition (< 80%) were measured in 83.33% of the individuals (Fig. 2). In contrast, this low level of inhibition was seen only in 13.33% and 10% of RR Aug Aug F2 and F4 insects, respectively. For LSU-S larvae only 3% of test insects showed levels of inhibition <80% (data not shown). In tests with OP-R larvae, mean levels of AChE inhibition were statistically similar to those of LSU-S, although low levels of inhibition (<80%) were measured in 16.67% of test insects. Poor correlations were calculated between levels of inhibition and profenofos susceptibility in analyses with all strains (r2=0.08) and MRS strains (r2=0.09), but were moderate for strains originated from RR collection (r2=0.59). Finally, AChE activity in field-collected and laboratory-selected strains were significantly higher than those measured from LSU-S larvae (data not shown).

Discussion

Early detection of insecticide resistance provides a basis for the management of resistant pest populations (Brewer and Trumble, 1991) and depends on development of rapid and sensitive tests. Rapid biochemical assays coupled with biological assays are potential tools for estimating both the intensity and frequency of resistance in the field (Brown and Brogdon, 1987). However, use of surrogate (non-insecticidal) substrates as monitoring tools is imprudent without establishing a relationship between enzyme activities with model substrates and insecticide susceptibility (Ibrahim and Ottea, 1995).

High frequencies of resistance to profenofos were evident among the field-populations tested and resistance was unstable in the absence of insecticides. Resistance to profenofos has been shown previously to be prevalent in cotton fields (Elzen, et al., 1994; Kanga et al., 1994) and was unstable in field populations of tobacco budworm (Kanga et al., 1995). In addition, frequencies of resistance and enzyme activities differed between the RR and MRS locations studied here. Such differences may be the result of more frequent insecticide applications at the RR loacation. In contrast, insects at the MRS location were collected from unsprayed plots of velvet leaf (MRS Jun), cotton (MRS Aug) and Dallas grass (MRS Sep), although effects of host plants or migration to and from neighboring fields that received insecticide applications were not evaluated.

Resistance to profenofos was highly correlated with EST activity toward a-NA suggesting an association between these enzymes and profenofos resistance. Similar relationships have been established in other insects between EST activities and resistance to OP (Devonshire and Moores, 1982; Pasteur and Georghiou, 1989; Xu and Brindley, 1993) and pyrethroid (Gunning et al., 1996) insecticides, although positive correlations are not always found (Carlini et al., 1994). Thus, this esterase assay has potential as a sensitive biochemical marker for monitoring profenofos resistance in field populations of *H. virescens*.

Activities of GST toward model substrates are often higher in OP-resistant than susceptible insects (reviewed in Clark, 1989). In previous studies where multiple strains were examined, activities toward DCNB were correlated with OP resistance in the tufted apple bud moth, *Platynota idaeusalis* (Carlini et al, 1994) and the diamondback moth, *Plutella xylostella* (Ku et al., 1994). In the present study, profenofos resistance was correlated with CDNB but not DCNB activity suggesting that the enzymes metabolizing these substrates have different identities and contributions to profenofos resistance. Further, correlations were higher for MRS than RR collections illustrating spatial variation in expression of GSTs associated with profenofos resistance.

Spatial variation also was evident in sensitivity of AChE to inhibition by chlorpyrifos oxon between insects from the two collection sites. Sensitivity of AChE to inhibition was poorly correlated in analysis including all strains. However, correlations between resistance and AChE inhibition levels for strains that originated from the RR Aug were greatest suggesting a linkage between reduced sensitivity of AChE and profenofos resistance at this (but not at MRS) site. Moreover, the correlation was better when frequencies (instead of mean levels) of AChE insensitivity and profenofos resistance were compared ($r2=0.69$) illustrating a possible contribution of a target site insensitivity to OP resistance as reported previously in *H. virescens* (Brown, 1991; Kanga and Plapp, 1994; Brown et al., 1996: Wolfenbarger, 1996), *Spodoptera littoralis* (Dittrich, et al., 1979), *Lygus hesperus* (Xu and Brindley, 1993).

Although levels of P450-MO activity towards PNA were elevated in all field-collected and OP-R larvae compared with LSU-S larvae, no correlation was found between these activities and frequencies of profenofos resistance indicating that measurement of this activity has no obvious toxicological significance. Activities of P450-MO have been reported to be associated with resistance to OP (Bull and Whitten, 1972; Reed, 1974) carbamate (Rose et al., 1995) and pyrethroid insecticides (McCaffery, et al. 1991; Ottea, et al., 1995) in *H. virescens.* In contrast, previous studies indicate no difference in P450-MO activity in OP-susceptible and resistant *H. virescens* (Gould and Hodgson, 1980). Activity of P450-MO toward PNA also was not found to be associated with cypermethrin resistance in *H. virescens* (Ibrahim and Ottea, 1995) although P450-MO plays an important role in cypermethrin detoxication (McCaffery et al., 1991; Ottea et al., 1995).

Summary

Profenofos resistance in *H. virescens* was found to be multifactorial involving both metabolic and target site mechanisms. Elevated levels of EST are associated with profenofos resistance in strains from both locations sampled and the utility of this assay as a biochemical marker for monitoring profenofos resistance in field populations of *H. virescens* is being explored further.

Acknowledgments

We thank Drs. Jerry Graves, Roger Leonard and Jonathan Holloway for providing insects for this study and Chad Prather and Jocelyn Chernetz for assistance with insect rearing. These studies were supported by funds from Cotton Incorporated and the Louisiana Agricultural Experiment Station (LAES). J. A. H. was supported by a stipend from the United Methodist World Hunger Fund.

References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide, J. Econ. Entomol. 18: 265.

Booth, J., E. Boyland, and P. Sims. 1960. Metabolism of polycyclic compounds: The conversion of naphthalene into a derivative of glutathione by rat liver slices, Biochem. J. 74: 117-122.

Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Chem. 72: 248-254.

Brown, T. M., K. Bryson, and G.T. Payne. 1982. Pyrethroid susceptibility in methyl parathion-resistant tobacco budworm in South Carolina, J. Econ. Entomol. 75: 301-303.

Brown, T. M. 1991. Resistant acetylcholinesterase of the tobacco budworm, *Heliothis virescens,* Rev. Pestic. Toxicol., 1: 185-195.

Brown, T. M., P. K. Bryson, F. Arnette, M. Roff, J. L. B. Mallett, J. B. Graves, and Stanley J. Nemec. 1996. Surveillance of resistant acetylcholinesterase in *Heliothis virescens*, In Molecular genetics and evolution of pesticide resistance, T. M. Brown (Ed) American Chemical Society, Washington, D. C., Vol. 645; pp137-148.

Bull, D. L. and J. J. Menn. 1990. Strategies for managing resistance to insecticides in Heliothis pests of cotton, IN:"Managing Resistance to Agrochemicals" Eds. M.B. Green and H. M. LeBaron and W.K. Moberg, American Chemical Society, washing ton D. C. 118-133.

Bull, D. L., and C. J. Whitten, 1972. factors influencing organophosphorus insecticide resistance in tobacco budworms, J. Agric. Food Chem. 20: 561.

Byrne, F. J. and A. L. Devonshire. 1993. Insensitive acetylcholinesterase and esterase polymorphism in susceptible and resistant population of the tobacco whitefly *Bemisia tabaci* (Genn.), Pestic. Biochem. Physiol. 45: 43-42.

Clark, A. G. 1989. The comparative enzymology of the glutathione *S*-Transferases from non-vertebrate organisms, Comp. Biochem. Physiol. B92: 419-446.

Devonshire, A. L. G. D. Moores and R. H. ffrench-Constant. 1986. Detection of insecticidee resistance to immunological estimation of carboxylesterase activity in *Myzus persicae* (Sulzer) and cross reaction of the antiserum with *Phordon humuli* (Schrank) (Hemiptera: Aphididae). Bull. Entomol. Res. 76: 97-107.

Dittrich, V., N. Luetkemeier, and G. Voss. 1979. Moncrotophos and profenofos: two organophosphates with a different mechanism of action in resistant races of *Spodoptera littoralis*, J. Econ. Entomol. 72:380-384.

Ellman, G. L., K. D. Courtney, V. Andres, R. M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7: 88.

Finney, D. J. 1971. Statistical Methods in Biological Assay, 2nd ed. Griffin Press, London.

ffrench-Constant, R. H. and R. T. Roush, 1990. Resistance detection and documentation: The relative roles of pesticidal and biochemical assays, In "Pesticide Resistance in Arthropods" (R. T. Roush and B. E. Tabashnik, Eds.), Chapman and Hall, New York, 110.

G. Gomori, 1953. Human esterases, J. Lab. Clin. Med. 42: 445.

Gould, F., and E. Hodgson, 1980. Mixed function oxidase and glutathione transferase activity in last instar *Heliothis virescens* larvae, Pestic. Biochem. Physiol. 13:34-40.

Grant, D. F., D. M. Bender, and B. D. Hammock, 1989. Quantitative kinetic assay for glutathione *S*-transferase and general esterase in individual mosquitoes using an EIA reader, Insect Biochem. 19, 741-751.

Grant, D. F., E. C. Dietze and B. D. Hammock, 1991. Glutathione *S*-transferase isozyme in *Aedes aegypti*: purification, characterization, and isozyme-specific regulation, Insect Biochem. 21, 421-433.

Graves, J. B. and D. F. Clower. 1975. Status of resistance in *Heliothis* species, Proceedings Beltwide Cotton Conferences. 142-145.

Hansen, L. G., and E. Hodgson. 1971. Biochemical characteristics of insect microsomes: *N* and *O*-demethylation, Biochem. Pharmacol. 20, 1569-1578.

Ibrahim, S. A. and J. A. Ottea.1995. Biochemical and toxicollgical studies with laboratory and Field populations of *Heliothis virescens* (F.). Pestic. Biochem. Physiol. 53: 116-128.

Jakoby, W. B. 1978. The glutathione S-transferases: A group of multifunctional detoxification proteins, Adv. Enzymol. 46: 383-414.

Kanga, L. H. B. and F. W. Plapp, Jr. 1994. Resistance monitoring and mechanism in the tobacco budworm to organophosphate, carbamate, and cyclodiene insecticides, Proceedings Beltwide Cotton Conferences. 810-815.

Kanga, L. H. B., F. W. Plapp, Jr., G. W. Elzen, M. L. Wall, and J. D. Lopez, Jr.,1995. Monitoring for resistance to organophosphorus , carbamate, and cyclodiene insecticides in tobacco budwornm adulst (Lepidopter: Noctuidae), J. Econ. Entomol. 88(5): 1144-1149.

Kinoshita, F. K., J. P. Frawley, and K. P. DuBois.1966. Quantitative measurement of induction hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rates, Toxicol. Appl. Pharmacol. 20, 1569-1578.

Kirby, M. L., R. J. Young, and J. A. Ottea. 1994. Mixed-function oxidase and glutathione S-transferase activities from field-collected larval and adult tobacco budworms, *Heliothis virescens* (F.), Pestic. Biochem. Physiol. 49,24-36.

Konno, T., E. Hodgson, and W. C. Dauterman. 1989. Studies on methyl parathion resistance in *Heliothis virescens* , Pestic. Biochem. Physiol. 33: 189-199.

Leonard, B. R., T. C. Sparks, and J. B. Graves. 1988. Insecticide cross-resistance in pyrethroid-resistant strains of tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 81: 1529-1535

McCaffery, A. R., R.T. Gladwell, El. Nayir, C.H. Walker, J. N. Perry, and M. J. Miles. 1991. Mechanisms of resistance to pyrethroids in laboratory and field strains of *Heliothis virescens* , Southwest. Entomol. 15**,** 143-158.

Oppenoorth, F. J., 1985. Biochemistry and genetics of insecticide resistance, in "Comprehensive Insect Physiology, Biochemistry and Pharmacology: (G. A. Kerkut and L. I. Gilbert, Eds.), Vol. 12, p 731, Pergammon Press, Oxford.

Oppenoorth, F. J., L. J. T. van der Pas, and N. W. H. Houx. 1979. Glutathione *S*-transferase and hydrolytic activity in a tetrachlorvinphos-resistant strain of housefly and their influence on resistance,Pestic. Biochem. Physiol. 11: 176-188.

Ottea, J. A., S. A. Ibrahim, A. M. Younis, B. R. Leonard and A. R. McCaffery. 1995. Biochemical and physiological

mechanisms of pyrethroid resistance in *Helitohis virescens* (F.), Pestic. Biochem. Physiol. 51, 117.

Reed, W. T. 1974. *Heliothis* larvae: variation in mixed function oxidase activity as related to insecticide tolerance, J. Econ. Entomol. 67: 150-152.

Sparks, T. C. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America, Bull. Entomol. Soc. Am. 27: 186-192.

Sparks, T. C., J. B. Graves, and B. R. Leonard. 1993. Insecticide resistance and the tobacco budworm: past, present and future, Rev. Pestic. Toxicol., 2: 149-183.

van Asperen, K. 1961. A study of housefly esterases by means of a sensitive colorimetric method, J. Insect Physiol., 5: 401-416.

Williams. 1996. M. R. Cotton insect losses. Proceedings Beltwide Cotton Conferences. 670-689.

Wolfenbarger, D. A. 1996. Tobacco budworm populations in cotton: heterogeniety of response to anticholinesterase inhibitors, Resist. Pest Manage. Newsl. 8, 47-48.

Xu, G., and W. A. Brindley. 1993. Structure of populations Lygus hesperus (Hemiptera: Miridae) with multiple resistance mechanisms to trichlorfon, J. Econ. Entomol. 86(6): 1656-1663.

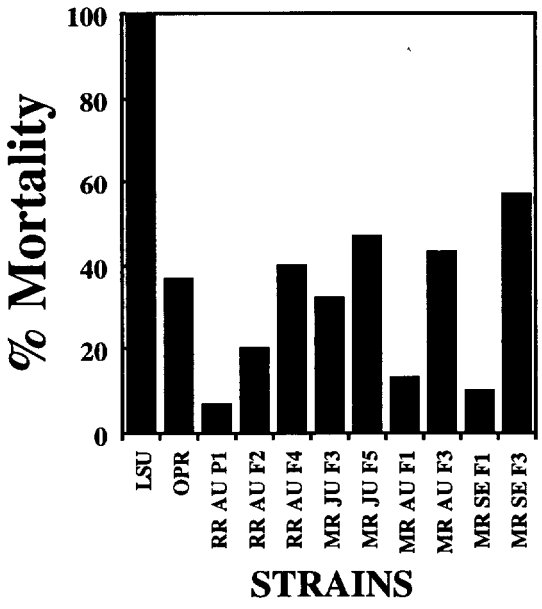

Fig. 1. Frequencies of profenofos resistance measured in field-collected and laboratory strains of H. virescens using a topically applied discriminating dose (15.3 µg profenofos/ larva; equivalent to 10X LD50 of LSU-S strain).

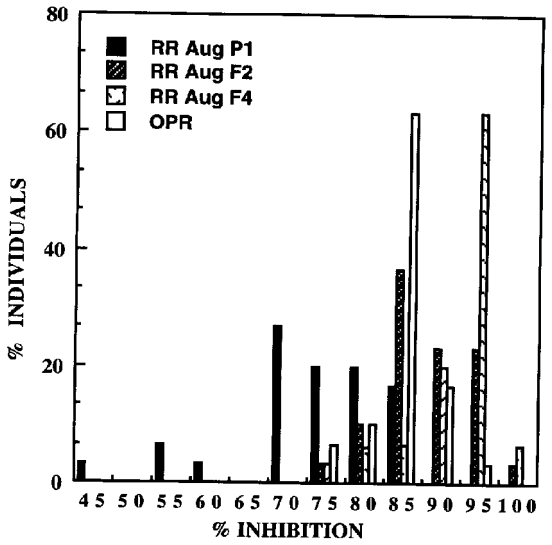

Fig. 2. Frequency of AChE inhibition by chlorpyrifos oxon in RR Aug and OP-R strains of the tobacco budworm. Concentration of inhibitor used corresponds to the I80 of AChE from the LSU-S strain.

Table 1. Levels of enzyme activities and inhibition of AChE by chlorpyrifos oxon in field-collected and laboratory strains of H. virescens

Strain	P450 MO	GST/ CDNB	GST/ DCNB	EST	AChE Inhibition
LSU-S	77.7^{d}	253^{e}	3.14^{d}	71.6^{d}	87.9^{ab}
OP-R	560^{bc}	836^{abc}	12.5^{abc}	160^{abc}	82.5^{ab}
RR Aug P1	676^{ab}	2370^{ab}	17.3^{a}	239^{a}	71.3^{c}
RR Aug F2	344^{c}	1150^{cd}	12.5^{abc}	206ab	85.6^{ab}
RR Aug F4	508^{bc}	1450^{bcd}	14.7^{ab}	161^{abc}	85.6^{ab}
MRS Jun F3	580^{abc}	2020^{abc}	10.1^{bc}	166^{abc}	88.1^{a}
MRS Jun F5	386^{c}	738^{d}	11.9^{abc}	134^{bc}	80.4^{b}
MRS Aug F1	406^{c}	2030^{abc}	13.3^{abc}	210^{ab}	86.7^{ab}
MRS Aug F3	805^{a}	1500^{bcd}	16.1a	42.0^{bc}	85.2^{ab}
MRS Sep F1	520^{bc}	2580^{a}	13.4^{abc}	200^{abc}	87.9^{a}
MRS Sep F3	463^{bc}	1180^{cd}	8.34^{c}	115^{c}	87.6^{bc}