INHERITANCE OF RESISTANCE TO ORGANOPHOSPHATE INSECTICIDES IN THE TOBACCO BUDWORM, *HELIOTHIS VIRESCENS* (F.) WITH SPECIAL REFERENCE TO THE CROSS RESISTANCE TO PYRETHROIDS

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Abstract

Heliothis virescens males were more effective in inheriting profenofos resistance than females. The degree of dominance of profenofos resistant alleles was (-0.22) and (+0.27) for the hybrid from crossing susceptible parent with female or male resistant parent, respectively. Resistance to profenofos in *H. virescens* larvae may be partially incompletely dominant sex linkage gene and incompletely recessive autosomal gene. There is a possibility of a partial cross resistance between organophosphates and pyrethroids through the incompletely recessive autosomal gene, the degree of dominance of cypermethrin resistance gene was (-0.18) or (-0.68), respectively for the hybrid from crossing resistant females or resistant males. Esterase activity measured toward 1-naphthyl acetate in larvae of tobacco budworm may be responsible about big part of profenofos detoxication ($r^2 = 0.69$), however play less role in pyrethroid hydrolysis ($r^2 = 0.32$). There was a specific band with Rm = 0.65±0.05 may be important for hydrolyzing profenofos, this band was more represented in resistance larvae. However no one of susceptible larvae expressed this specific band. Pupae of *H. virescens* may not be an optimum stage to characterize the biological and biochemical expressions of resistance, esterase activities measured in pupae did not correlate ($r^2 = 0.077$) with those measured in larvae. Topical application technique with *H. virescens* adults did not express resistance to profenofos or cypermethrin.

Introduction

The tobacco budworm, *Heliothis virescens* (F.) was first known as a cotton pest in 1934 (Folsom, 1936), and is one of the most damaging pests of cotton in the United States (Wolfenbarger, *et. al.* 1981 and Sparks, 1993). Together with the bollworm, *Heliocoverpa zea, H. virescens* has caused more than $20 million in total crop losses and costs of control in 1998 (Williams, 1999).

The inefficiency of insecticides on *H. virescens* is essentially related to the fast development of insecticide resistance (Sparks, 1981 and Sparks *et. al.* 1993). Over the past 40 years, resistance in *H.virescens* has developed toward chlorinated hydrocarbons (Lingren and Bryan, 1965; Lowry, 1966 and Graves, *et. al.* 1967), organophosphates (OPs) and carbamates (Graves and Clower, 1971 and Harris, 1972), and finally pyrethroids after their extensively use in cotton fields during the late 1970's (Wolfenbarger *et. al.* 1981). At present, *H. virescens* is being effectively managed with transgenic cotton that expresses a toxin from *Bacillus thuringiensis* (Bt), however, the efficacy of these toxins also is threatened as three Bt-resistant strains of *H. virescens* have been developed by selection in the laboratory (Gould, 1991 and Gould *et. al.* 1995). In addition, Elzen, (Elzen, 1997) found one field strain of *H. virescens* that was significantly resistant to foliar formulations of *B. thuringiensis* among strains collected in Mississippi in 1993 through 1995.

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There are three mechanisms associated with OP resistance in *H. virescens* (Szeicz *et. al.* 1973; Whitten and Bull, 1978; Konno *et. al.* 1989; Konno *et. al.* 1990; Brown and Bryson, 1992; Kanga and Plapp, 1995 and Harold and Ottea, 1997). Reduced cuticular penetration appears to be a minor mechanism in resistance to OP (Kanga and Plapp, 1994) and pyrethroids (Ottea *et. al.* 1995). In contrast, enhanced metabolic detoxication involving esterases (ESTs) is a major resistance mechanism to the OPs, methyl parathion (Konno *et. al.* 1989) and profenofos (Harold and Ottea, 1997) and pyrethroid-resistance in *H. virescens* (Goh *et. al.* 1995 and Shan, 1997). Reduced target site sensitivity was found to be an additional mechanism in OP resistant *H. virescens* (Harold and Ottea, 1997).

Study the cross-resistance is an essential requisite before using an insecticide to control any insect species. The development of resistance to an insecticide by *H. virescens* may be accompanied by varying levels of cross-resistance to other insecticides, even with non-similar mechanisms of action (Leonard *et. al.* 1988). Resistance to pyrethroid insecticides developed relatively quickly because of the background of cross-resistance from chlorinated hydrocarbon (Sparks *et. al.* 1988). Also the variation in susceptibility of *H. virescens* to pyrethroids before their widespread was as a result of the pre-existing extensive use of organophosphates, in addition, with pyrethroid-resistant *H.virescens,* different levels of resistance to OPs and carbamates were measured (Plapp *et. al.* 1987 and Campanhola and Plapp, 1989).

The number of genes and the degree of dominance of resistance alleles are essential factors affecting the development of insecticide resistance. However, the inheritance of resistance differs depending upon the mechanism responsible, even in the same insect species. For example, resistance to methyl parathion in *H. virescens* appeared to be influenced by a single, major, autosomal gene of incomplete dominance that controls levels of the major enzyme systems involved in the detoxication of methyl parathion (Whitten, 1978). In contrast, a later study indicates the role of sex linkage and additive gene action in the inheritance of methyl parathion resistance in this insect species (Wolfenbarger *et. al.* 1982). Differences between the findings of these reports may be related to the expression of different resistance mechanisms in the two strains studied.

Studying the genetic control of insecticide resistance in this insect species became one of the most important subjects (Bloch and Wool, 1994). Genetic or biochemical markers are required to distinguish the effects of minor genes involved in insecticide resistance (Roush and Daly, 1990). For example the need for electrophoretic markers in *H. virescens* has been emphasized for inheritance studies (Heckel *et. al.* 1988). The objective of this study was to measure the inheritance of resistance to profenofos in larvae, pupae and adults of *H. virescens*. The susceptibilities of resistant and susceptible parents and their progeny toward profenofos and cypermethrin were measured to study the inheritance of organophosphate resistance and to further pyrethroid insecticides.

Materials and Methods

Chemicals

Technical grade (89%) profenofos (*O*- (4-bromo-2-chlorophenyl)-*O*-ethyl-*S*-propylphosphorothioate was donated by Novartis (Greensboro, NC). Technical grade cypermethrin (96.2%) was obtained from FMC Corporation (Princeton, NJ). Acrylamide, ethylenediaminetetraacetic acid (EDTA) and tris (hydroxymethyl) aminomethane (Tris) were purchased from Gibco BRL, (Grand Island, NY). N, N, N, N-tetramethylethylene-diamine (TEMED), bis acrylamide, ammonium persulfate and Commassie Brilliant Blue (R-250) were obtained from Amresco (Solon, OH). Bovine serum albumin (fraction 5), Fast Blue RR salt, B-naphthyl acetate (B-NA) and 1-naphthyl acetate (1-NA) were purchased from Sigma Chemical Company (ST. Louis, MO). Fast Blue B salt was purchased from Aldrich

Chemical Company (Milwaukee, WI). Sucrose and bromophenol blue were purchased from J. T. Baker Chemical Co. (Phillipsburg, N. J.).

Insects

The susceptible strain (LSU-S) was established from a field collection made from cotton in 1977 (Leonard *et. al.* 1988) and was reared in the laboratory without intentional exposure to insecticides. The pyrethroid-resistant (Pyr-R) strain was collected from the Red River Research Station (Bossier City, LA) during 1995 and was selected in the laboratory with cypermethrin (Shan, 1997) was also used in comparison. An organophosphates resistant laboratory strain (OP-R) was established by selecting larvae from a field collection made during 1995 (Harold and Ottea, 1997) at the Red River Research Station, (Bossier City, LA).

Insecticide Susceptibility

Susceptibility of *H. virescens* to profenofos and cypermethrin was measured in fifth stadium (day 1) larvae following application of 1 µl of acetone containing varying doses of profenofos (0.5 to 40 µg) or cypermethrin (0.01 to 2.6 µg) onto the thoracic dorsum. Control larvae were treated with acetone only. The dose-mortality response of larvae was measured with at least 10 doses of each chemical (10 larvae/dose) and replicated thrice. Treated larvae were held in 1-oz cups with diet and maintained at 27°C, 70±5% relative humidity, and a photoperiod of 14:10 (light: dark) hr. Mortality was based on failure of treated larvae to pupate. Treated and untreated larvae were observed until pupation or death and numbers of pupae were counted. Mortality data were corrected (Abbott, 1925), analyzed by probit analysis (Finney, 1971) using a microcomputer-based program, SAS, 1985 (SAS, 1985).

Susceptibility of *H. virescens* pupae to profenofos was measured following application of 1 µl of acetone containing varying doses (0.5 to 10 µg) of profenofos onto the thorax. The dose-mortality response of pupae was measured with at least 5 doses (10 larvae/dose) and replicated five times. Treated pupae were held in 1-oz cups and maintained at 27°C, 70±5% relative humidity, and a photoperiod of 14:10 (light: dark) hr. Treated and untreated pupae that were observed until adult eclosion (12-15 days), then the number of adults was counted. Mortality was based upon failure of adult emergence. Data were corrected (Abbott, 1925), analyzed by probit analysis (Finney, 1971) using a microcomputer-based program, SAS, 1985 (SAS, 1985).

Susceptibility of *H. virescens* adults to profenofos was measured following application of 0.5 µl of acetone containing varying doses of profenofos (0.05 to 2.0 µg) onto the left eye. The dose-mortality response of adults was measured with at least 5 doses (10 adults/dose) and replicated five times. Treated adults were held in 1-oz cups and maintained at 27°C, 70±5% relative humidity, and a photoperiod of 14:10 (light: dark) hr. Mortality of treated and untreated adults was recorded 24 hr after treatment. An adult was considered dead if it failed to maintain upright posture after being dropped onto a hard surface (Ottea *et al.*, 1999). Data were corrected (Abbott, 1925), analyzed by probit analysis (Finney, 1971) using a microcomputer-based program, SAS, 1985 (SAS, 1985)

Insecticide Selection and Genetic Crossing

For the current study, the OP-R strain was selected with the profenofos inhibitory dose of 80% pupae formation that was 6.8, 12.9, 22.6 and 29.3 µg. /larva for the original OP-resistant strain and the three successive generations of selection (OP-R, OP-R1, OP-R 2 and OP-R 3, respectively). The LD_{80f} for the fourth generation of selection (OP-R 4) was 29.9 µg/larva.

Pupae of LSU-S and OP-R4 were sexed (Butt and Cantu, 1962) and separated until adult eclosion. Twenty males or females from each strain were put in cardboard cartons covered with cotton gauze with the same number from the opposite sex of the other strain. Pupae from the F1 progeny of crossing resistant females with susceptible males were sexed and adult males were backcrossed with females from the OP-R4 strain.

Tissue Preparation

Tissue homogenates from individual larvae, pupae, and adults were used as enzyme source for measuring EST activities toward 1-NA. Individual larvae were dissected and the digestive system was removed. The opened hemocoel was rinsed with ice-cold buffer (0.1 M sodium phosphate, pH 7.0) and fat bodies were removed by gentle scraping and aspiration using a Pasteur pipette. A larval fat body, an entire pupa or adult abdomen was homogenized in an all-glass homogenizer containing ice-cold buffer (0.1 M sodium phosphate, pH 7.0). The volumes of buffer used for homogenizing were 600 µl (for fat body) or 1 ml (for adult or pupa). Homogenates were centrifuged at 10,000 g for 15 min at 4°C. The resulting supernatants were filtered through glass wool then held in ice and used in enzyme assays within 30 min of preparation.

Biochemical Assay

Activity of ESTs toward 1-NA was measured using the assay of Gomori (Gomori, 1953) with modifications (van Aspern, 1961; Grant *et. al.* 1989 and Ibrahim and Ottea, 1995). Reaction mixtures in individual wells of a microtiter plate were made from 30 µl of fat body homogenate and 220 µl of substrate dye solution. The fat body homogenate per individual well contained 0.05 tissue equivalent and 0.06±0.01 mg protein for individual larva; 0.03 tissue equivalent and 0.45±0.12 mg protein for pupa; 0.03 tissue equivalent and 0.25±0.06 mg protein for adult. The substrate dye solution was prepared by adding 0.013 gm. of 1-NA dissolved in 100 µl acetone to 25 ml phosphate buffer (pH 7.0) containing 0.018 gm Fast Blue B salt, and then stirred and filtered using Whatman #3 filter paper. The final concentrations of 1-NA and Fast Blue B salt in the reaction were 2.04 and 1.18 mM, respectively. The reaction mixtures were incubated at 25°C, and the rate of change in absorbance during the initial 10 min was measured at 450 nm using a Thermomax microplate reader (Molecular Devices, Palo Alto, CA). Data were corrected for non-enzymatic activity using incubation without protein as the control. Changes in Optical density were converted to nmol/min/assay using the extinction coefficient 9.25 mM^{-1} 250 μl^{-1} (Grant *et. al.* 1989).

Native polyacrylamide gel electrophoresis (PAGE) was used to visualize EST from individual larvae, pupae and adults using a vertical electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) and 5% polyacrylamide (Gunning *et. al.* 1996). Protein concentrations in homogenates of individual fat body, pupa or adult abdomen were adjusted to contain 200 µg protein in 50 ul phosphate buffer pH 7.0 containing 0.1% Triton X-100 which was mixed with 5 µl of 6X tracking dye (0.25% bromophenol blue and 40% sucrose in double-distilled water). Electrophoresis occurred in electrode buffer consisting of 100 mM Tris, 2.4 mM EDTA, and 100 mM boric acid (pH 8.0) at a constant voltage (150 V) until the dye marker was within 1 cm of the gel base. After electrophoresis, gels were stained in darkness at 25°C with 200 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.5 mM 1-NA and B-NA and 0.2% Fast Blue B Salt for 1 hr. Gels were destained in water and fixed in 5% acetic acid. Relative mobility (Rm) was calculated by dividing the migration distance of specific band from the origin to the center of the band by the migration distance of the marker from the origin.

Protein concentrations were measured from diluted homogenates by the method of Bradford (Bradford, 1976). Absorbency was measured at 595 nm using a Thermomax microplate reader (Molecular Devices, Palo Alto, CA). Data were corrected using incubations without protein as the control.

Statistical Analysis

Data were subjected to one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test ($P \leq 0.05$) using a microcomputer-based program (SAS, 1985). Linear regressions between

enzyme activities and susceptibility toward profenofos and cypermethrin were estimated using the methods of least squares.

Results

Insecticide Resistance

Larvae of fifth stadium (day 1) from the pre-selected strain (OP-R), profenofos-selected strain (OP-R4), a pyrethroid resistant strain (Pyr-R), a susceptible strain (LSU-S) and the F1 progeny of crossing OP-R4 and LSU-S were compared in their susceptibility toward profenofos. The comparison based upon the medium inhibitory doses of pupae formation (LD_{50}) and the level of resistance relative to LSU-S larvae. Susceptibility of OP-R4 significantly decreased after four generation of selection. Larvae from OP-R strain was 4.3-fold resistant (LD_{50} = 4.76 µg/larva), however the resistant level of OP-R4 was 19.5-fold (LD_{50}= 21.49 µg /larva). The hybrids of crossing OP-R4 and LSU-S parents were located between the two parents regarding the medium lethal doses of profenofos. The LD_{50} were 7.22 or 3.49 µg /larva from the hybrid of crossing OP-R4 males or females with the opposite sex from LSU-S, respectively. But when males from hybrid of OP-R4 female parent were backcrossed with females from OP-R4 strain, resistance level increased to 5.5-fold. The frequency of resistance (The LD-P line figures not shown) revealed that about 14% of the hybrid from crossing resistant males with susceptible females responded to profenofos doses ≤ 2.5 μg/larva. However, 30% of those hybrid were resistant responded the same way as resistant parent to the does of ≥17 μg/larva. The rest of individuals (56%) were in the border zone (>2.5-<17 μg/larva) between susceptible and resistant parent. As for the hybrid from the opposite crossing, 30% of individual larvae responded to the range of doses of LSU-S parent (≤ 2.5 μg/larva); no individual larva was in the zone of resistant parent (≥17 μg/larva). The degree of dominance (Stone, 1968) of resistant alleles was -0.22 or +0.27 for the hybrid of crossing susceptible parent with females or males from resistant parent, respectively. It was also evident that larvae from Pyr-R strain that did not expose to any OP-insecticide treatment since 1995, was 8.7-fold resistant toward profenofos.

Susceptibility of larvae from profenofos-selected (OP-R4), susceptible (LSU-S) and their F1 progeny toward cypermethrin was compared with larvae from a pyrethroid resistant (Pyr-R) strain. The OP-R4 larvae had the same resistance level toward cypermethrin as those from a Pyr-R (Table. 1). The LD_{50}'s were 0.93 and 0.89 µg. /larva, respectively, with resistance ratio averaging 11.9- and 11.4-fold, respectively. Larvae from the pre-selected strain (OP-R) did not show any level of resistance toward cypermethrin, with, LD_{50} value (0.09 µg. /larva) was not significantly different as compared with LSU-S larvae (0.08 μg/larva). The resistance level of larvae from the F1 progeny of crossing either the resistant males or females with the susceptible opposite sex did not reach three-fold. The degree of dominance of cypermethrin resistant alleles (Stone, 1968) was -0.18 and -0.68, respectively for the hybrid from crossing susceptible parent with resistant females or resistant males, respectively. There was a moderate correlation between the susceptibility of larvae from the seven tested strains toward profenofos and cypermethrin (r^2 = 0.64). The resistance of OP-R4 larvae toward profenofos (19.5-fold) has been accompanied with high level of resistance toward cypermethrin (11.9-fold). The contrast was also evident as the pyrethroid resistant larvae with 11.4-fold resistance level toward cypermethrin exhibited 8.7-fold resistant toward profenofos. However larvae from the F1 progeny of crossing either resistant males or females with susceptible ones did not show significant levels of resistance toward cypermethrin.

Levels of profenofos resistance (based upon the medium inhibitory doses of adult eclosion) on the pupae (day-1) from profenofos-selected strain (OP-R4) and a pyrethroid resistant strain (Pyr-R) were relatively higher than those from the other tested strains (Table 2). However the resistance ratio did not exceed 2-fold relative to those from the susceptible strain (LSU-S). The LD_{50} (95% CL) values ranged between 3.95(2.4-6.13) and 6.6 (3.88-8.75) for OP-R4 and the Pyr-R strains, respectively. Susceptibility of pupae from the OP-R4 strain did not significantly differ with those from the F1 progeny of crossing either resistant males or females with the susceptible ones. Moreover, pupae from the pre-selected strain (OP-R) were not significantly more susceptible than those from the susceptible strain with LD_{50} values (95% CL) were 1.13 (0.9-5.02) and 2.75 (1.9-3.5) µg/pupa, respectively.

In topical application assay, the sensitivity of adults from susceptible (LSU-S) and selected (OP-R4) strains as well as the pyrethroid resistant (Pyr-R) strain toward profenofos did not significantly differ. The resistance ratio was 1.97, 1.82 and 2.29-fold relative to susceptible strain for adults from the OP-R, OP-R4 and the Pyr-R strains, respectively. In general, larvae were about 14- to 33-fold more resistant than adults. For example, OP-R4 Larvae expressed 19.5-fold resistant to profenofos, however, the resistance level of adults was less than 2-fold. Moreover, the toxicity responses of adults did not significantly differ before or after selection (OP-R and OP-R4).

Esterase Activities

Esterase activity (EST) measured in larvae toward 1-naphthyl acetate (1-NA) was significantly elevated after four generations of selection (OP-R4), as compared with that measured in the pre-selected larvae (OP-R). Mean EST activity was 88.6 nmol min^{-1} mg $protein^{-1}$ in larvae from the OP-R strain and increased to 173.7 nmol min^{-1} mg $protein^{-1}$ in OP-R4 larvae (Table 3). Mean EST activity in susceptible larvae (LSU-S) was significantly less (43.5 nmol min^{-1} mg $protein^{-1}$) as compared with OP-R and OP-R4 larvae. The hybrid of crossing OP-R4 males with LSU-S females expressed significantly greater EST activity (142.2 nmol min^{-1} mg $protein^{-1}$) than those from the opposite crossing (61.6 nmol min^{-1} mg $protein^{-1}$). In addition, activity measured in larvae from the F1 progeny of crossing OP-R4 males with LSU-S females did not significantly differ with those measured in larvae from the OP-R4 parent. However, larvae from F1 progeny of crossing resistant parent females and susceptible parent males had statistically the same esterase activity (61.6 nmol min^{-1} mg $protein^{-1}$) as those of susceptible parent (43.5 nmol min^{-1} mg $protein^{-1}$.). Larvae from the F1 generation of back crossing males of this strain with females from the OP-R4 revealed significantly greater activity (108.3 nmol min^{-1} mg $protein^{-1}$) as compared with that measured in susceptible parent (61.6 nmol min^{-1} mg $protein^{-1}$). , but was statistically lower than those measured in resistant parent (173.7 nmol min^{-1} mg $protein^{-1}$). Pyrethroid resistant larvae showed relatively high esterase activity (161.1 nmol min^{-1} mg $protein^{-1}$) that was not significantly different as compared with that measured in the OP-R4 larvae. Esterase activities measured in larvae from the seven tested strains moderately (r^2 = 0.69) correlated with the corresponding toxicity responses toward profenofos, but weakly correlated (r^2 = 0.32) with those toward cypermethrin.

Frequency histograms of esterase activity measured in individual larvae from susceptible strain (LSU-S), profenofos selected strain (OP-R4) and the F1 progeny of crossing them are graphed in Figure.1. Larvae from LSU-S parent as well as, the F1 progeny of crossing LSU-S males with OP-R4 females are shifted to the left. About 80% of the individuals had esterase activity ≤ 75-nmol min^{-1} mg $protein^{-1}$. However, 20% of the two tested populations shifted to the middle portion with esterase activity ranging 100-125 nmol min^{-1} mg $protein^{-1}$. In contrast, more than 50% of larvae from the F1 progeny of crossing OP-R4 males with LSU-S females shifted toward the resistant parent with esterase activity ≤150 nmol min^{-1} mg $protein^{-1}$. In addition, about 40% of the individuals were in the borderline between resistant and susceptible parent, sharing about 20% of susceptible parent the moderate activity of 75 -125 nmol min^{-1} mg $protein^{-1}$. About 28% of LSU-S larvae expressed EST activity ≤ 25 nmol min^{-1} mg $protein^{-1}$, however, not one of any of the tested strains expressed this lower activity.

Esterase activity toward 1-naphthyl acetate (1-NA) was also measured in the pupae from susceptible (LSU-S) and profenofos-selected (OP-R4) strains as well as the F1 generation of crossing them. The OP-R4 pupae expressed the greatest activity with mean value did not significantly differ with that measured in the F1 progeny of crossing LSU-S males with OP-R4 females (39.8 and 36.8 nmol min^{-1} mg $protein^{-1}$, respectively). Both expressed significantly greater EST as compared with the F1 progeny from the opposite crossing and LSU-S pupae (23.6 and 25.3 nmol min^{-1} mg. $Protein^{-1}$, respectively). Esterase activities measured in the pupae from the six tested populations weakly correlated with the corresponding bioassay data toward profenofos (r^2= 0.413). Moreover they did not correlate with that measured in larvae (r^2 = 0.077). Larvae from F1 progeny of crossing LSU-S males with OP-R4 females exhibited lower esterase activity as compared with that measured in larvae from the opposite crossing (61.6 and 142.2 nmol min^{-1} mg $protein^{-1}$, respectively). But, the contrast was evident when EST activities measured in pupae were compared (36.8 and 23.6 nmol min^{-1} mg $protein^{-1}$, respectively).

Adults from the profenofos selected strain (OP-R4) expressed relatively the greatest EST activity (27.4 nmol min^{-1} mg $protein^{-1}$). However, it did not significantly differ from those measured in their F1 progeny from the susceptible parent (LSU-S). Mean EST activity measured in the hybrid of crossing OP-R4 males with LSU-S females was not significantly greater than that measured from those of the opposite crossing (21.6 and 18.7 nmol min^{-1} mg $protein^{-1}$, respectively). However, both expressed statistically the same esterase activity measured in the susceptible and resistant parents. Mean EST activity measured in adults from LSU-S and the Pyr-R strains (17.59 and 15.9 nmol min^{-1} mg $protein^{-1}$) was significantly lower than that measured in the OP-R4 adults (27.4 nmol min^{-1} mg $protein^{-1}$). Mean EST activities measured in pupae and larvae from LSU-S, OP-R4 and the hybrid of crossing them were strongly correlated (r^2 = 0.87). However when Pyr-R strain was included, the correlation was weak (r^2 = 0.244). The Pyr-R strain did not keep the same trend regarding esterase activity in larvae and adults. Esterase activity measured in larvae was about 3-fold greater than that measured in those from susceptible strain. However, adults of this strain exhibited statistically the same esterase activity as compared with susceptible strain. Moreover, Adult bioassay data in topical application assay did not correlate (r^2 = 0.017) with the corresponding esterase activities measured.

Electrophoretic Analysis

The pre-selected strain (OP-R) larvae that exhibited about 4.3-fold resistant toward profenofos and about 2-fold more esterase activity relative to susceptible strain (LSU-S), expressed 2-8 EST bands. More than 70% of the OP-R individual larvae missed one band in the position of Rm = 0.657 (Figure. 2). The pattern of EST bands in individual larvae from profenofos-selected strain (OP-R4) and Susceptible strain (LSU-S) was compared (Figure 3). All of OP-R4 larvae expressed specific band with Rm value = 0.627, however not one of LSU-S larvae had this band. Furthermore, OP-R4 larvae missed another band in the position of Rm = 0.395. In addition, about 70% of larvae from the hybrid of crossing OP-R4 males with LSU-S females expressed specific band in the position of Rm = 0.675 and all of them missed another band in the position of Rm = 0.313 (Figure 4). In contrast approximately, all individuals from opposite crossing expressed this upper band (Rm = 0.313) but, only 33% of them expressed the lower band (Rm = 0.675). This means that specific EST bands in the position with Rm ranging 0.65 ± 0.05 may be responsible in profenofos resistant in *H. virescens* larvae.

Discussion

Knowing the pattern of inheriting insecticide resistance is essential for predicting the length of time an insecticide will be effective in controlling an insect species. The inheritance of profenofos resistance in *H. virescens*. was more effective through resistant males than resistant female. Larvae from the F1 progeny of crossing resistant males with susceptible females were 6.6-fold resistant toward profenofos. However, those from the opposite crossing were relatively less resistant (RR = 3.5-fold relative to susceptible parent). but when males of that hybrid backcrossed with females from profenofos-selected strain, the resistance level increased to 5.5-fold. The degree of dominance (Stone, 1968) of profenofos resistance alleles was (-0.22) and (+0.27) for the hybrid of female and male resistant parent, respectively. This means that profenofos resistant alleles in *H. virescens* females can identified as an incompletely recessive. However males expressed incompletely dominant gene that was responsible about great part of profenofos resistance in *H. virescens*. Resistance to profenofos in *H. virescens* larvae may be partially due to the sex linkage gene. The possibility of sharing additive autosomal gene must be considered. This may explain why: (1) about 14% of the hybrid from crossing resistant males with susceptible females were susceptible responded to profenofos doses of ≤ 2.5 μg/larva, and (2) the hybrid of crossing resistant females with susceptible males was 3.5-fold more resistant than the susceptible parent. Number of genes and the degree of dominance of resistance alleles affected the development of resistance (Georghiou and Taylor, 1986; Roush and Daly, 1990). Even in the same insect species, different strains may express different mechanisms of resistance and the inheritance of resistance differs depending upon the mechanism responsible. For example, methyl parathion resistance in *H. virescens* appeared to be influenced by a single, major, autosomal gene of incomplete dominance (Whitten, 1978), however the role of sex linkage and additive gene action was also considered (Wolfenbarger *et. al.* 1982). Recently Harold, (Harold, 1998) reported the role of sex linkage gene in the inheritance of profenofos resistance in *H. virescens*. Reported here confirmed the role of both sex linkage (incompletely dominant gene) and additive autosomal gene (incompletely recessive) in profenofos resistance in *H. virescens.*

In many cases, *H. virescens* develops resistance to an insecticide even with dissimilar mechanisms of action (Leonard *et. al.* 1988). Report here revealed that the profenofos-resistant *H. virescens* (OP-R strain) that, was kept in the laboratory since 1995, without exposure to any insecticide treatment expressed significant level of resistance toward profenofos (4.3-fold relative to susceptible strain). However, this strain was susceptible to the toxicity action of cypermethrin (1.1-fold). After four generations of selection with the LD_{80} of profenofos, the level of resistance toward profenofos increased to 19.5-fold and has been accompanied with 11.4-fold resistance to cypermethrin. Cross-resistance toward cypermethrin from previous use of organophosphates was also reported (Leonard *et. al.* 1988). However larvae from the hybrid of crossing either resistant males or females with the opposite sex from the susceptible parent expressed significantly different levels of resistance toward profenofos, but statistically the same lower level of resistance toward cypermethrin regardless of the sex of resistant parent. In general, there was a moderate correlation between the susceptibility of larvae from the seven tested strains toward profenofos and cypermethrin (r^2 = 0.64). Resistance toward cypermethrin in *H. virescens* seemed to be controlled by autosomal gene with incomplete recessivity, the degree of dominance of resistance gene (according to the formula by Stone, (Stone, 1968) was (-0.18) or (-0.68), for the hybrid from crossing resistant females or resistant males parent, respectively with the opposite sex from the susceptible parent. The possibility of a partial cross resistance between organophosphates and pyrethroids through the incompletely recessive autosomal gene could be a reason why: (1) profenofos-selected *H. virescens* (OP-R4) larvae expressed high level of resistance toward profenofos (19.5-fold) and crossed about 60% of those mechanisms to cypermethrin (11.9-fold) and (2) Why there were insignificant differences in susceptibility toward cypermethrin between the hybrid from crossing either resistant males or resistant females with the opposite sex from susceptible parent. These results are in agreement with the finding of Roush and Luttrell, 1987; Payne *et al.* 1988 and Watson and Kelly, 1991, they reported that permethrin resistance in *H. virescens* was inherited as a single, major, incompletely recessive

autosomal factor. With 11.4-fold cypermethrin resistant *H. virescens* (Pyr-R), a level of 8.7-fold resistance to profenofos was measured. Researchers (Plapp *et. al.* 1987 and Companhola and Plapp, 1989) reported that pyrethroid resistant *H. virescens*, expressed varying levels of resistance to organophosphates and carbamates.

Esterase activity measured toward 1-naphthyl acetate in larvae of tobacco budworm may be responsible about major part of profenofos detoxification in the larvae of this insect species ($r^2 = 0.69$), however play less role in pyrethroid resistance ($r^2 = 0.32$). Profenofos-resistant larvae (OP-R4) as expressed the greatest level of resistance toward profenofos (19.5-fold) expressed also the greatest level of esterase activity (173.7 nmol min^{-1} mg $protein^{-1}$). Also more than 60% of individual larvae from the hybrid of crossing resistant males with susceptible females were in the same zone of resistant parent regarding esterase activity. About 30% of those hybrid were resistant to the doses of $\leq$ 17 ug./larva sharing with the resistant parent, profenofos resistance zone (LD-P line figures did not show). However 90% of the hybrids from the opposite crossing were in the zone of susceptible parent regarding EST activity. In addition 30% of those individuals shared with susceptible parent the same zone of susceptibility toward profenofos (responded to $\leq$ 2.5 ug./larva). In contrast no one was in the zone of resistant parent ($\geq$ 17 ug./larva). Enhanced metabolic detoxification involving esterases is considered the major mechanism in the organophosphate, methyl parathion (Konno *et. al.* 1990) and profenofos (Harold and Ottea, 1997) and pyrethroid-resistance in *H. virescens* (Goh *et. al.* 1995 and Shan, 1997). There was a specific band with Rm = 0.65±0.05 which may be important for hydrolyzing profenofos, this band was represented in 100, 70, 33 and 0.0% of OP-R4, F1 of crossing resistant males with susceptible females, the F1 of the opposite crossing and susceptible larvae, respectively. Similar band with Rm = 0.68 was observed in field collected *H. virescens* larvae (Harold, 1998), however susceptible laboratory stains did not express this band.

Pupae of *H. virescens* may not be an optimum stage to characterize the biological and biochemical expressions of resistance. Level of resistance did not exceed 2-fold with any of tested strains. In addition, the level of resistance toward profenofos in larvae from OP-R and OP-R4 were about 10.5-, 13.9-fold greater than that in pupae. Moreover esterase activities measured in pupae did not correlate ($r^2 = 0.077$) with those measured in larvae. Larvae from the F1 progeny of crossing resistant males with susceptible females exhibited greater esterase activity than those from the opposite crossing, however, the contrast was evident when esterase activities measured in pupae were compared. On the other hand larvae from the original colony were 4.3-fold more resistant toward profenofos than those from susceptible one, but pupae of this strain were insignificantly more susceptible to profenofos than those from susceptible strain.

Topical application technique with *H. virescens* adults did not express the resistance to profenofos or cypermethrin. The sensitivity of adults from the susceptible and the profenofos-selected as well as the pyrethroid-resistant strains did not significantly differ. The medium lethal doses of profenofos on larvae were about 14- to 33-fold greater in comparison with adults. Moreover, the correlation was weak ($r^2 = 0.35$). In addition, adult bioassay data in topical application assay did not correlate ($r^2 = 0.017$) with the corresponding esterase activities measured. In spite of that, Esterase activity measured in adults from selected and susceptible strains as well as the F1 progeny of crossing them were strongly correlated ($r^2 = 0.87$) with those measured in larvae, but not with those measured in pupae ($r^2 = 0.288$). However when esterase activity measured in larvae from pyrethroid resistant strain was included, the correlation was very weak ($r^2 = 0.24$). The pyrethroid resistant strain did not keep the same order regarding esterase activity measured in larvae and adults. Activity measured in larvae was about 3-fold greater than that measured in those from susceptible strain, however, adults of this strain exhibited insignificantly less esterase activity than those of susceptible one.

In conclusion, Profenofos resistance in *H. virescens* seems to be inherited via incompletely dominant sex linkage gene and an additional incompletely recessive autosomal gene. Cross-resistance toward cypermethrin is through the autosomal gene. Pupae are not the optimum stage of studying the biochemical expression of resistance toward organophosphate in *H. virescens.* Topical application is not an accurate technique of expressing resistance of *H. virescens* adults toward profenofos.

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Table 1. Susceptibility of larvae to profenofos and cypermethrin in susceptible and profenofos-selected strains of *H. virescens*.

	Profenofos			Cypermethrin		
Strain	[1]LD_{50} (95% CL)	Slope (SE)	[2]RR	[1]LD_{50} (95% CL)	Slope (SE)	[2]RR
LSU	1.10 (0.69-1.52)	6.38 (0.31)	--	0.08 (0.052-0130)	3.40 (0.36)	--
OP-R	4.76 (2.99-6.05)	5.57 (0.43)	4.3	0.09 (0.048-0.184)	3.76 (0.56)	1.1
OP-R4	21.49 (17.30-26.50)	5.95 (0.32)	19.5	0.93 (0.694-1.51)	1.87 (0.16)	11.9
F1 (R4fXSm)	3.49 (2.90-5.30)	2.77 (0.18)	3.2	0.22 (0.162-0.43)	3.62 (0.27)	2.8
F1 (R4mX LSf)	7.22 (5.98-10.10)	2.03 (0.17)	6.6	0.12 (0.088-0.24)	4.83 (0.39)	1.5
F1BC[f1 (R4fXSm) mXR4f]	6.06 (4.50-9.32)	1.49 (0.11)	5.5	0.21 (0.099-0.49)	1.99 (0.19)	2.7
PYR-R	9.54 (11.60-14.36)	3.29 (0.27)	8.7	0.89 (0.52-1.26)	2.09 (0.31)	11.4

[1]measured in topical bioassays with fifth instar larvae that were counted dead if they failed to pupate.
[2]Resistance ratio (RR)=LD_{50} of tested strain/LD_{50} of susceptible strain

Table 2. Susceptibility of pupae and adults to profenofos and cypermethrin in susceptible and profenofos-seleclted strains of *H. virescens*.

	Pupae			Adult		
Strain	[1]LD_{50} (95% CL)	Slope (SE)	[2]RR	[3]LD_{50} (95% CL)	Slope (SE)	[2]RR
LSU	2.75 (1.90-3.50)	3.47 (0.19)	---	0.35 (0.52-1.09)	3.91 (0.36)	---
OP-R	1.13 (0.90-5.02)	1.33 (0.25)	0.41	0.691 (0.45-1.02)	4.46 (0.39)	1.97
OP-R4	3.95 (2.40-6.13)	2.54 (0.33)	1.43	0.636 (0.38-1.11)	2.66 (0.19)	1.82
F1 (R4fXSm)	5.10 (2.60-7.19)	4.07 (0.67)	1.85	0.25 (0.09-0.64)	1.47 (0.11)	0.71
F1 (R4mXLSf)	3.75 (1.85-5.61)	4.17 (0.56)	1.36	0.56 (0.09-1.06)	1.15 (0.14)	1.60
PYR-R	6.59 (3.88-8.75)	2.87 (0.39)	2.40	0.80 (0.69-1.23)	0.87 (0.19)	2.30

[1]measured I topical bioassays with pupate. Pupa was counted dead if they failed to emerge within 15 days post-treatment.
[2]Resistance ratio (RR)=LD_{50} of tested strain/LD_{50} of susceptible strain.
[3]measured in topical bioassays with adults based upon 24 hr morality.

Table 3. [1]Esterase activities measured in the laboratory susceptible and profenofos-selected larvae, pupae and adults of tobacco budworm *H. virescens* and f1 progeny of crossing of susceptible and OP-resistant strains.

	1-napthol product (nmol $min.^{-1}$ $mg.protein^{-1}$) in		
Strain	Larvae	Pupae	Adult
LSU	43.51 (26.6)c	25.33 (11.71)b	17.5 (2.10)b
OP-R	88.635 (34.22)b	[2]ND	ND
OP-R4	173.71 (47.1)a	39.80 (16.57)a	27.39 (8.5)a
F1 (R4fXSm)	61.59 (32.26)c	36.84 (13.16)a	18.65 (5.19)ab
F1 (R4mXL Sf)	142.19 (82.09)ab	23.58 (14.84)b	21.55 (4.98)ab
F1BC[f1 (R4fXSm) mXR4f]	108.29 (86.01)b	ND	ND
PYR-R	161.084 (90.51)a	ND	15.85 (5.16)b

Mean values in the same column followed by the same letter are not significantly different (ANOVA, Tukey-Kramer; $P \leq 0.05$)
[1]Esterase activities as nmol/min/mg.protein (± SD) measured toward 1-naphthyl acetate.
[2]Not determined.

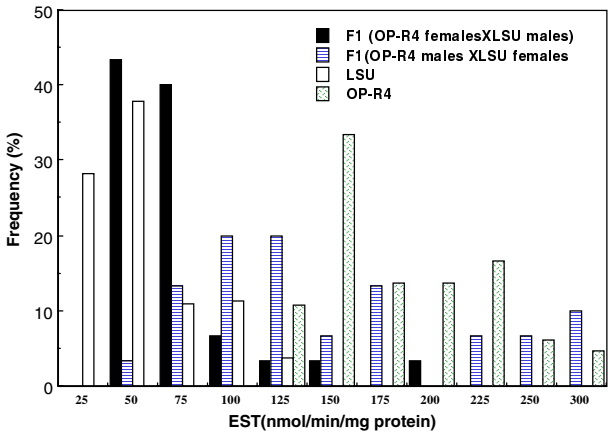

Figure 1. Frequency distribution of EST activity (1-NA hydrolysis min-1 mg protein-1) in larvae of LSU susceptible strain, Profenofos-selected colony and the F1 progeny of crossing them.

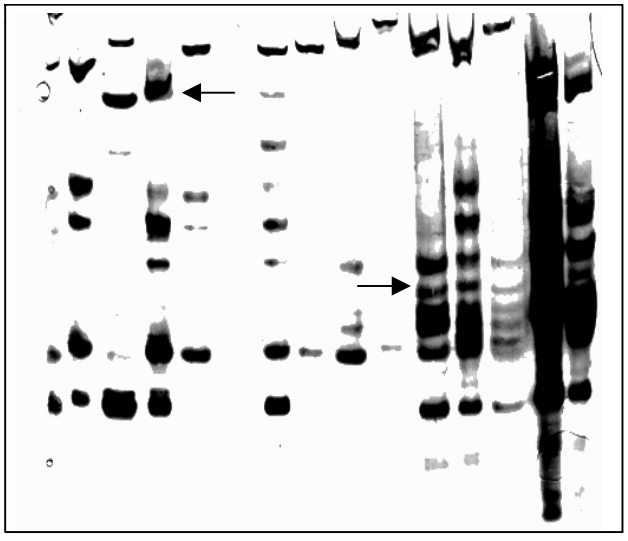

Figure 2. Polyacrylamide gel stained for EST activity in 14 individuals of fifth stadium (day one) larvae from OP-R strain of *H. virescens* (just before selection.)

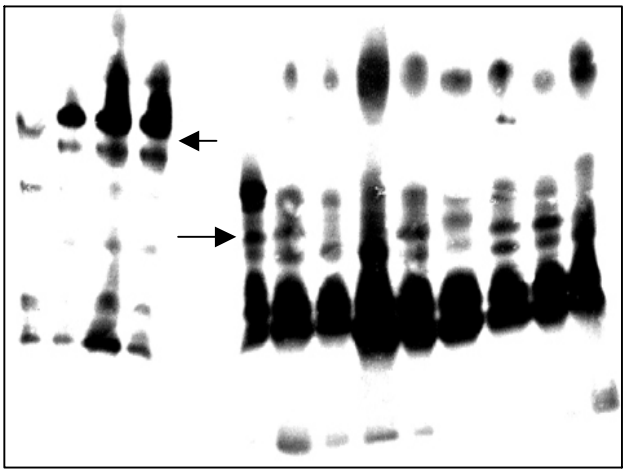

Figure 3. Polyacrylamide gel stained for EST activity in 4 individuals of fifth stadium (day one) larvae from LSU-susceptible strain of *H. virescens*, (to the left); and 9 individuals from Profenofos-selected strain, (to the right).

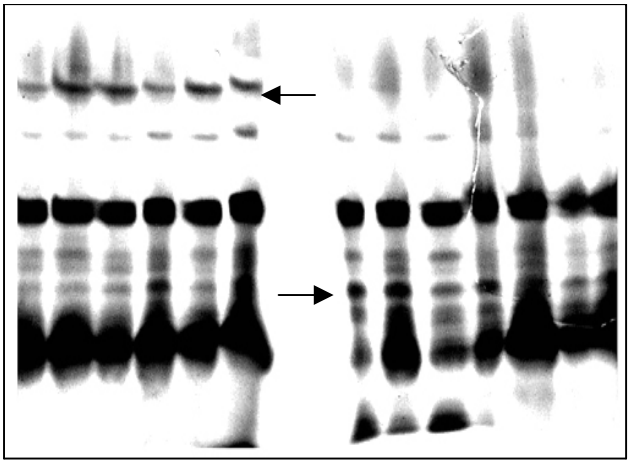

Figure 4. Polyacrylamide gel stained for EST activity in 6 individual larvae of F1 progeny from crossing resistant *H. virescens* females with susceptible males, (to the left) and 7 individuals from the opposite crossing, (to the right).