BIOCHEMICAL MECHANISMS OF PYRETHROID RESISTANCE IN CYPERMETHRIN-SELECTED *HELIOTHIS VIRESCENS*

S.A. Ibrahim[1], A. M. Younis[1] and J. A. Ottea[2]
[1]Dept. of Plant Protection, Fac. of Agriculture, Minia University, Minia, Egypt.
[2]Dept. of Entomology, LA State Univ. Ag. Center, Baton Rouge, LA

Abstract

Mechanisms of pyrethroid resistance in cypermethrin-selected tobacco budworms, *Heliothis virescens* were studied using a pharmacokinetic assay and radiolabelled cypermethrin. Selection of larvae and adults resulted in increased levels of resistance, reduced rates of penetration and increased excretion of cypermethrin metabolites. Although both life stages expressed the same mechanisms, development of resistance was faster in colonies selected as adults rather than larvae. Results from *in vitro* assays using model substrates for mixed-function oxidase, esterase and glutathione *S*-transferase activities were not correlated with the increased metabolism measured in pharmacokinetic assays suggesting that activity toward these model substrates did not reflect that of enzymes involved in cypermethrin metabolism and resistance.

Introduction

Pyrethroid insecticides have been the central component of chemical strategies to manage populations of the tobacco budworm, *Heliothis virescens*. Because of extensive use of these compounds, resistance to pyrethroids has evolved in populations of tobacco budworms throughout the mid-South (Leonard *et al*., 1987; Roush and Luttrell, 1987; Campanhola and Plapp, 1989; Graves *et al*., 1990; 1993; Clower *et al.*, 1991; Martin *et al.*, 1994).

A more detailed understanding of biochemical and physiological mechanisms of resistance is essential to develop rational countermeasures for insecticide resistance. The majority of previous research describing resistance mechanisms in *H. virescens* has been directed toward the crop-damaging larval stage of this insect (Little *et al*., 1989; McCaffery *et al*., 1989, 1991; Ottea *et al*., 1995). However, selection among adult populations also occurs in the field. In addition, adults (not larvae) are used to detect and monitor resistance in field populations of this insect (Plapp *et al*., 1987). Finally, resistance frequencies in field populations vary between larvae and adults suggesting that expression of resistance mechanisms may differ between these two life stages (Forrester *et al*., 1993; Ottea *et al*., 1995).

The primary objective of this study was to identify and compare mechanisms of pyrethroid resistance in tobacco budworms from colonies of insects that were selected with cypermethrin as either larvae or adults. Results from biological and biochemical studies show that increased levels of resistance was accompanied with enhanced metabolism and reduced cuticular penetration in both life stages.

Materials and Methods

Chemicals: Cypermethrin (*trans* isomer, 98+ % pure) was supplied by FMC Corporation (Princeton, NJ). Radiolabelled cypermethrin was provided by ICI Agrochemicals (Bracknell, Berkshire, U.K.). 3-phenoxybenzoic acid (PBA), glucose-6- phosphate, glucose-6- phosphate dehydrogenase, reduced nicotinamide adenine dinucleotide phosphate (NADPH), *p*-nitroanisole (PNA), bovine serum albumin (fraction 5), reduced glutathione (GSH), and alpha-naphthyl acetate (a-NA) were purchased from Sigma Chemical Co., Inc. (St. Louis, MO). Phenylthiourea (PTU), trichloroacetic acid (TCA), potassium chloride (KCl), 3,4-dichloronitrobenzene (DCNB), 1-chloro-2,4-dinitrobenzene (CDNB) and Fast Blue B salt were purchased from Aldrich Chemical Company (Milwaukee, WI).

Insects: Eggs and newly hatched larvae were collected at random on July 12, 1992 near Harlingen (HAR), TX from a cotton field with a history of heavy insecticide use and control difficulties. Insects were reared on a pinto bean - based artificial diet (Leonard *et al.*, 1988) and held at 27°C , LD 14:10 and 70% relative humidity. Larvae were identified as *H. virescens* using mandible morphology as the criterion (Oliver and Chapin, 1981). Adult insects were reared in one gallon ice-cream containers covered with nylon mesh and provided with a 10% sucrose: water solution as a carbohydrate source. An insecticide-susceptible strain of *H. virescens* (LSU) that has been reared for over 15 years without insecticide exposure (Leonard *et al*., 1988) was used as reference strain for biochemical and toxicological assays.

Bioassays and Selection: Fifth instar larvae (day 1) and newly emerged adults (<1 day old) were used for both biochemical and biological assays. Larvae were segregated in the final day of the fourth instar using head capsule slippage as the criterion. The following day, insects that had completed molting and commenced feeding were treated on the second thoracic dorsum with 1 μl of acetone containing varying concentrations of cypermethrin. Mortality was defined as the absence of coordinated movement within 30 seconds after being prodded with a blunt probe and was scored at 24 hrs. posttreatment. For bioassays with adults, insects were held for 24 hrs. inside

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1054-1059 (1996)
National Cotton Council, Memphis TN

15 ml. scintillation vials that had been pretreated with varying concentrations of cypermethrin in acetone (Plapp *et al.*, 1987). Adults were considered dead if they failed to maintain upright posture after being dropped 2 feet onto a hard surface. Bioassay data were corrected for control mortality using Abbott's (1925) formula , then subjected to probit analysis using the method of Finney (1971). The average body weight (mg. $\pm$ SD) of insects was 184.6 $\pm$ 13.8 and 133.1 $\pm$ 12.03 for larvae and adults, respectively. For selection experiments, the parent, HAR population was reared without insecticide pressure until the fourth laboratory generation. At this time, the colony (HAR F4) was divided into two subcolonies: the first was selected as fifth instar larvae and the second as 1-2 day old adults. In tests with successive generations, insects were exposed to cypermethrin in topical or treated vial bioassays at concentrations corresponding to the LD- or LC_{80} for larvae or adults, respectively. Survivors from these assays were used for biochemical assays or were maintained in culture.

Biochemical Assays: Tissue homogenates from a single larva (fifth instar, day 1) were used as enzyme source for assays of mixed-function oxidase (MFO; midgut), glutathione *S*-transferase (GST; fat body) or esterase (EST; fat body) activities. In tests with adults (one day old), abdomens were homogenized and used for all assays (Ibrahim and Ottea, 1995).

Measurements of MFO activities toward PNA were made using the procedure of Kinoshita *et al*. (1966) as modified by Hansen and Hodgson (1971) and Ibrahim and Ottea (1995). Incubations without substrate were used as non-enzymatic blanks. Reactions were initiated by adding PNA (in ethanol, 0.35 mM final concentration) and were incubated at 25°C for 40 min., then stopped by adding TCA (2.5% or 5% final concentration for larval or adult assays, respectively) with vigorous mixing. The absorbance of reaction products was measured at 405 nm in microplates using a Thermomax microplate reader (Molecular Devices). Absorbance values were corrected for non-enzymatic activity and converted to pmoles *p*-nitrophenol formed/ minute using an experimentally derived "extinction coefficient" of 10.41 $mM^{-1}200\mu l^{-1}$ (Kirby *et al.*, 1994).

The activities of GST toward DCNB or CDNB were measured using the techniques of Booth *et al.* (1960) or Jakoby (1978), respectively, with modifications (Grant *et al*., 1989; Kirby *et al.*, 1994). Reaction mixtures were incubated in flat-bottom microtiter plates (Costar, Cambridge, MA) at 25°C and the rate of change in optical density (OD) at 340 nm during the initial 10 min. of the reaction was measured and corrected for non-enzymatic metabolism using incubation without protein as the control. The corrected OD/min. was converted to nmol/min. using the experimentally derived "extinction coefficients" of 8.5 or 10.9 $mM^{-1}300\mu l^{-1}$ for DCNB or CDNB, respectively (Grant *et al.*, 1989; 1991).

Measurements of EST activity toward a- NA were made using the assay of Gomori (1953) with modifications (van Asperen, 1962; Grant *et al*. 1989; Ibrahim and Ottea 1995). Reactions were incubated at 25°C in flat-bottom microtiter plates (Costar, Cambridge, MA) and absorbance during the initial 10 min. of the reactions was measured at 450 nm. Results were corrected for non-enzymatic metabolism and converted to nmol/min using the experimentally derived "extinction coefficient" of 9.25 $mM^{-1}250\mu l^{-1}$ (Grant *et al*.. 1989).

Enzyme activities were expressed as product formed min^{-1}mg $protein^{-1}$. Protein concentrations of enzyme homogenates were estimated by the method of Bradford (1976) using bovine serum albumin as the standard. Data were expressed per mg protein to facilitate comparisons between homogenates prepared from different tissues of larvae and adults of the same strain. Mean activities (±SE) were based on duplicate assays of 23-46 larvae or 21-82 adults and were compared between strains using analysis of variance followed by Tukey's Multiple Comparison Test ($P \leq 0.05$).

Pharmacokinetic Assay: Penetration, metabolism and excretion of *trans* -cypermethrin were measured *in-vivo* using the method of Little *et al*.. (1989) with modification (Ottea et al., 1995). Fifth instar larvae and adults from LSU, Harlingen F4 (S0) and successive generations of the larval- and adult-selected colonies were used for conducting this assay. Larvae were treated topically with 0.0167 μg of *trans*-cypermethrin containing ^{14}C-*trans*- cypermethrin (53 mCi/mmol; ca. 5000 dpm, phenoxybenzyl-alcohol labeled, 97+% pure) in acetone. This dose was equivalent to the LD_{25} of the reference LSU strain. For tests with adults, insects were incubated for 5 min. at 0°C, then treated in the left eye with 1 μl of an acetone solution containing 0.023μg of *trans*-^{14}C-cypermethrin. This dose is equivalent to the LD_{10} for adults from the reference LSU strain. Treated insects were held for 24 hrs, and surviving insects were used for subsequent analysis.

Penetration of cypermethrin into surviving larvae and adults was quantitated by acetone rinse followed by liquid scintillation counting. Metabolism and excretion of cypermethrin were quantitated after extraction of metabolites from carcass homogenates and excreta in methanol followed by thin-layer chromatography (Ottea et al., 1995). Values presented as percent excretion represent the sums of metabolites extracted and identified from insect feces, and are expressed relative to penetrated dose. Data are expressed as means based on analysis of at least 30 larvae or adults from each colony. Mean values were compared using a one-way analysis of variance and a Tukey's Multiple Comparison Test ($P<0.05$).

Results and Discussion

Cypermethrin Resistance: Susceptibility to cypermethrin was measured in both larvae and adults from laboratory-susceptible (LSU) and -resistant (S0) colonies of tobacco budworm. In addition, insects from laboratory colonies that were selected for 5 to 9 generations as either larvae (S5L- S9L) or as adults (S5A- S9A) were tested.

In bioassays with larvae, significant levels of resistance (4.8- to 12.7-fold) were measured from cypermethrin-selected colonies (Table 1). For both larval- and adult-selected colonies, levels of resistance increased following each successive round of selection. In addition, resistance levels were similar between the two selected colonies when individual generations were compared (e.g. S5L vs. S5A).

Levels of resistance were greater in adults than larvae from the two, cypermethrin-selected colonies (Table 2). As in tests with larvae, resistance increased with selection: whereas the parent (S0) adults were 2.9-fold resistant to cypermethrin, resistance ratios (relative to LSU adults) ranged from 15.5- to 29.2-fold after selection. In addition, there were no significant differences in resistance ratios between selected colonies when compared within the same generation. Thus, in both larvae and adults, the rate of development and magnitude of cypermethrin resistance were similar regardless of whether larvae or adults were selected.

Penetration and Metabolism of Cypermethrin: Delayed penetration and enhanced metabolism appear to be major mechanisms of pyrethroid resistance in the cypermethrin-selected strains. Levels of penetration decreased with selection in both adults and larvae from the two selected strains (Figures 1 and 2). Whereas penetration was greater in larvae from larval-selected colonies as compared with those from adult-selected colonies, decreases were similar between larvae and adults, regardless of the life stage selected. Finally, decreases in penetration measured during continuous selection were associated with increased resistance. Correlations between cypermethrin penetration and susceptibility were moderate for larval -selected insects (r^2= 0.73 and 0.82 for larvae and adults, respectively) but less strong for adult-selected insects (r^2= 0.57 and 0.68 for larvae and adults, respectively).

Metabolism and excretion of cypermethrin in larvae from selected colonies did not significantly increase until the third generation (S3) of selection (Figures 3 and 4). However, with subsequent selection from S3 to S5, enhanced metabolism was evident with similar patterns measured for larvae from the two selected colonies. Increased metabolism and resistance to cypermethrin in larvae from the two colonies were strongly correlated, (r^2= 0.97 or 0.85 for larval- and adult-selected larvae, respectively). Similarly, metabolism and excretion of cypermethrin increased markedly in adults from both colonies (Figures 5 and 6) and was correlated (r^2= 0.93 and 0.87 for larval- and adult-selected larvae, respectively) with reduced susceptibility to cypermethrin.

Enzyme Activities Toward Model Substrates: The activities of three groups of enzymes typically involved in insecticide metabolism and resistance were measured and compared with bioassay data from the pyrethroid-susceptible and -resistant laboratory strains. In tests with larval homogenates, MFO and GST activities were equivalent or greater in pyrethroid-resistant than those from the susceptible LSU strain (Table 3). However, in no case were activities in either larval- or adult-selected strains significantly greater than those of the parent, S0 strain. Thus, there were no significant correlations between the enzyme activities measured and resistance to cypermethrin. Similarly, larval EST activity was variable among resistant strains but was not significantly different from activity measured in LSU homogenates (Table 3). In addition, expression of larval EST activity and cypermethrin resistance were poorly correlated (r^2=0.01; data not shown). Finally, variability in enzyme activities from adults (Table 4) was not correlated ($r^2 < 0.20$; data not shown) with levels of resistance measured in bioassays.

Acknowledgments

We thank Dr. Dan Clower for supplying the field insects used in this study. This research was supported by the Louisiana Agricultural Experiment Station.

References

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

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 the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72: 248-254.

Campanhola, C., and F. W. Plapp. 1989. Pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae): Insecticide bioassays and field monitoring, J. Econ. Entomol. 82: 22-28.

Clower, D. F., B. Rogers, W. Mullins, D. Marsden, C. A. Staetz, B. J. Monke, J. Phelps, and G. Certain. 1991. Status of *Heliothis/Helicoverpa* resistance to pyrethroids in US

cotton, In" Proc. Beltwide Cotton Prod. Res. Conferences" National Cotton Council, Memphis, TN, pp. 739-742.

Finney, D. J. 1971. "Probit Analysis", 3 rd. Ed., Cambridge University Press, NY., 333 pp.

Forrester, N. W., M. Cahill, L. J. Bird, and J. K. Layland. 1993. Pyrethroid resistance: Field resistance mechanisms, Bull. Entomol. Res. Supp. Ser. 1: 54-61.

Gomori, G. 1953. Human esterases. J. Lab. Clin. Med. 42, 445-453.

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 isozymes in *Aedes aegyptii* : purification, characterization, and isozyme-specific regulation. Insect Biochem. 21: 421-433.

Graves, J. B., B. R. Leonard, S. Micinski, and E. Burris. 1990. Status of pyrethroid resistance in tobacco budworm in Louisiana, In "Proc. Beltwide Cotton Prod. Research Conf.", National Cotton Council, Memphis, TN, pp. 216-219.

Graves, J. B. , B. R. Leonard, S. Micinski, E. Burris, S. H. Martin, C. A. White, and J. L. Baldwin. 1993. Monitoring insecticide resistance in tobacco budworm and bollworm in Louisiana, In "Proc., Beltwide Cotton Res. Conf.", National Cotton Council, Memphis, TN, pp. 788-794.

Ibrahim, S. A., and J. A. Ottea. 1995. Biochemical and toxicological studies with laboratory and field populations of *Heliothis virescens* (F.). Pestic. Biochem. Physiol. 45:116-128.

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

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 budworm, *Heliothis virescens* (F.). Pestic. Biochem. Physiol. 49: 24-36.

Leonard, B. R., J. B. Graves, T. C. Sparks, and A. M. Pavloff. 1987. Susceptibility of bollworm and tobacco budworm larvae to pyrethroid and organophosphate insecticides. In "Proc. Beltwide Cotton Prod. Res. Conf.", National Cotton Council, Memphis, TN, pp. 320-324.

Leonard, B. R. , J. B. Graves, T. C. Sparks, and A. M. Pavloff. 1988. Variation in resistance of field populations of tobacco budworm and bollworm (Lepidoptera: Noctuidae) to selected insecticides. J. Econ. Entomol. 81: 1521-1528.

Little, E. J., C. H. Walker and A. R. McCaffery. 1988. A comparison of the pharmacokinetics of ^{14}C-trans cypermethrin in a resistant and a susceptible strain of *Heliothis virescens*. Proc. Brighton Crop Protect. Conf. Pests and Diseases 1: 427-432.

Little, E. J., A. R. McCaffery, C. H. Walker, and T. Parker. 1989. Evidence for an enhanced metabolism of cypermethrin by a monooxygenase in a pyrethroid resistant strain of tobacco budworm (*Heliothis virescens* F.). Pestic. Biochem. Physiol. 34:58-68

Martin, S. H., J. B. Graves, B. R. Leonard, E. Burris, S. Micinski, J. A. Ottea, and G. Church. 1994. Evaluation of insecticide resistance and the effect of selected synergists in tobacco budworm. In "Proc. Beltwide Cotton Res. Conf.", National Cotton Council, Memphis, TN, pp. 818-823.

McCaffery, A. R., E. J. Little, R. T. Gladwell, G. J. Holloway, and C. H. Walker. 1989. Detection and mechanisms of resistance in *Heliothis virescens.* In "Proc. Beltwide Cotton Prod. Res. Conf.", National Cotton Council, Memphis, TN. p. 207.

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

Oliver, A. D., and J. B. Chapin. 1981. Biology and illustrated key for the identification of twenty species of economically important Noctuid pests. LA Agric. Exp. Sta. Res. Bull. 733:3-26.

Ottea, J. A., S. A. Ibrahim, A. M. Younis, R. J. Young, B. R. Leonard, and A. R. McCaffery. 1995. Biochemical and physiological mechanisms of pyrethroid resistance in *Heliothis virescens* (F.). Pestic. Biochem. Physiol. 51:117-128.

Plapp, F. W., G. M. McWhorter, and W. H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm. In "Proc. Beltwide Cotton Prod. Res. Conf.", National Cotton Council, Memphis, TN. pp. 324-326.

Roush, R. T. and R. Luttrell. 1987. The phenotypic expression of pyrethroid resistance in *Heliothis* and implications for resistance management. In "Proc. Beltwide Cotton Prod. Res. Conf.", National Cotton Council, Memphis, TN. pp. 31-33.

van Asperen, K. 1962. A Study of housefly esterases by means of a sensitive colourmetric method. J. Insect. Physiol. 5: 401-416.

Table 1: Toxicity of cypermethrin to fifth instar larvae from laboratory susceptible (LSU), resistant (S0) and cypermethrin-selected strains of *H. virescens* .

Strain	Slope	LD_{80} µg/larva	95% F.L.
LSU	3.04	0.247	0.184- 0.438
So(HF4)	2.22	0.668	0.562- 0.899
S5L.	2.05	1.184	0.924- 1.742
S7L.	1.79	1.809	1.310- 3.146
S9L.	1.93	2.383	1.825- 3.469
S5A.	2.30	1.477	1.147- 2.180
S7A.	2.47	1.540	0.958- 5.580
S9A.	1.44	3.136	2.760- 8.890

Table 2: Toxicity of cypermethrin to moths from laboratory susceptible (LSU), resistant (S0) and cypermethrin-selected strains of *H. virescens* .

Strain	Slope	LD_{80} (ppm)	95% F.L.
LSU	1.49	3.34	1.52- 4.19
So(HF4)	2.99	9.69	8.91- 10.79
S5L.	N.D.*	N.D.	N.D.
S7L.	3.98	36.18	30.45- 47.67
S9L.	4.33	63.64	55.24- 83.66
S5A.	4.83	28.50	23.47- 38.49
S7A.	5.63	53.16	47.27- 63.67
S9A.	4.89	97.50	76.58- 166.9

*N.D. = not determined

Table 3: Larval MFO, GST and EST activities from laboratory susceptible (LSU), field-collected (S0) and cypermethrin-selected strains of *H. virescens*[1].

Strain	MFO[2]	GST/ CDNB[3]	GST/ DCNB[3]	EST[3]
LSU	131.8 B	108.3 B	4.3 C	111.8 AB
So(HF4)	286.2 AB	242.3 AB	13.6 A	N.D[4]
S5L.	163.9 B	471.1 A	8.7 AB	125.0AB
S7L.	392.9 A	252.6 AB	7.4 ABC	94.1 B
S9L.	125.9 B	224.1 AB	9.5 AB	130.0 AB
S5A.	158.9 B	472.6 A	7.9 ABC	153.9 A
S7A.	236.5 B	252.1 AB	6.5 BC	108.3 AB
S9A.	130.01 B	269.6 AB	5.1 BC	92.8 B

[1]Means within a column followed by the same letter are not significantly different (P≤0.05; Tukey's Multiple Comparison).
[2]pmoles product formed/min/tissue equivalent
[3] nmoles product formed/min/tissue equivalent
[4] N.D.= not determined

Table 4: Adult MFO, GST and EST activities from laboratory susceptible (LSU), field-collected (S0) and cypermethrin-selected strains of *H. virescens*[1].

Strain	MFO[2]	GST/ CDNB[3]	GST/ DCNB[3]	EST[3]
LSU	110.8 A	165.8 B	4.3 A	96.3 A
So(HF4)	N.D.[4]	N.D.	N.D.	N.D.
S5L.	90.1 A	193.6 AB	2.8 B	59.3 C
S7L.	140.6 A	105.9 C	4.4 A	60.03 CD
S9L.	119.7 A	172.8 B	3.3 AB	49.3 D
S5A.	78.2 A	223.5 A	3.5 AB	76.7 B
S7A.	152.8 A	73.2 C	4.1 A	63.6 C
S9A.	110.5 A	196.7 AB	3.9 A	60.3 CD

[1]Means within a column followed by the same letter are not significantly different (P≤0.05; Tukey's Multiple Comparison).
[2]pmoles product formed/min/tissue equivalent
[3] nmoles product formed/min/tissue equivalent
[4] N.D.= not determined

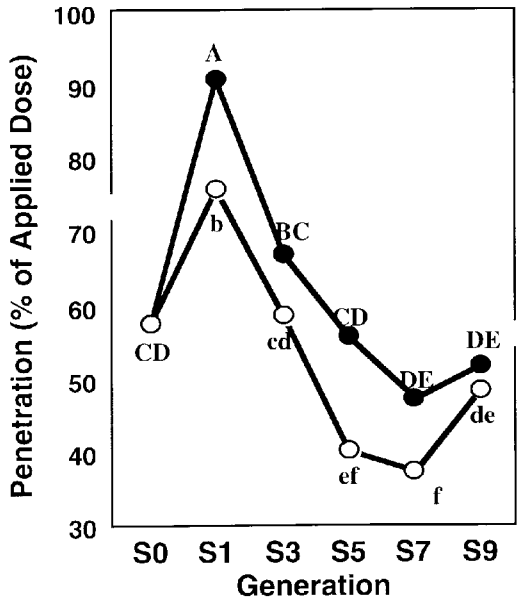

Figure 1: Penetration of cypermethrin into larval *H. virescens* from colonies selected as larvae (closed circles) or adults (open circles).

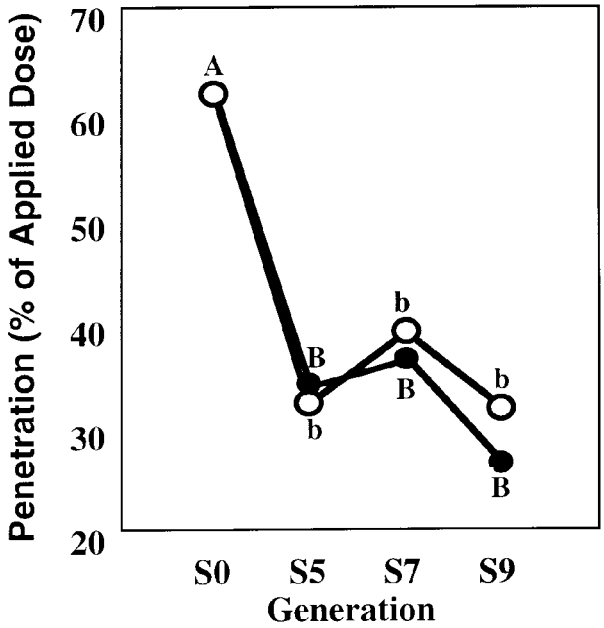

Figure 2: Penetration of cypermethrin into adult *H. virescens* from colonies selected as larvae (closed circles) or adults (open circles).

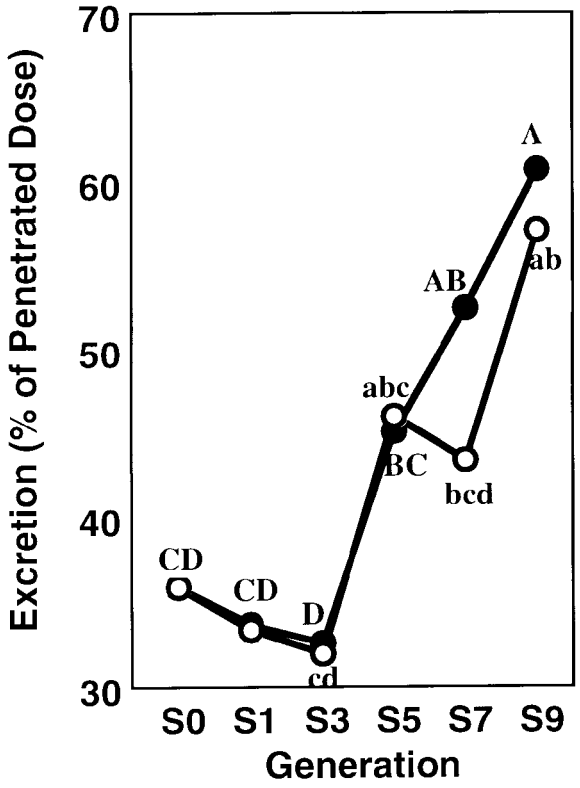

Figure 3: Levels of cypermethrin metabolites extracted from feces of larval *H. virescens*. Strains tested were selected as either larvae (closed circles) or adults (open circles).

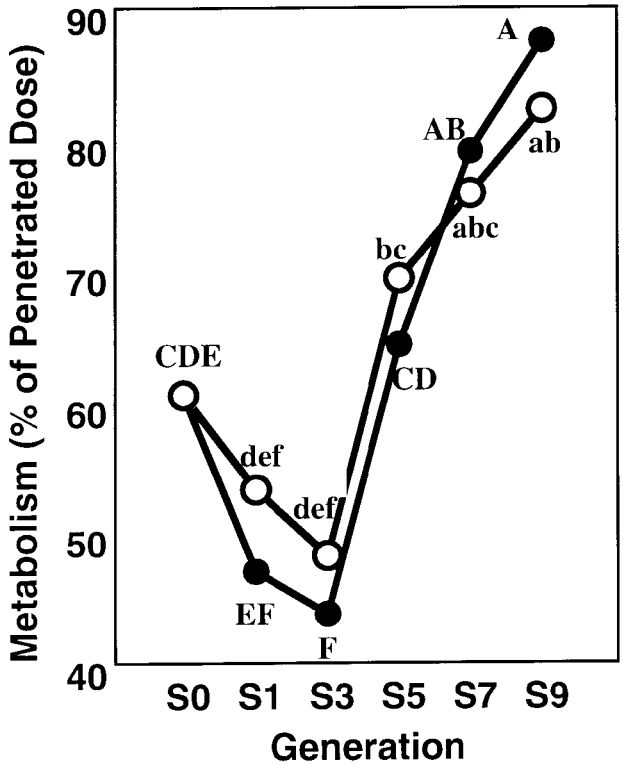

Figure 4: Levels of cypermethrin metabolites extracted from feces and carcasses of larval *H. virescens*. Strains tested were selected as either larvae (closed circles) or adults (open circles).

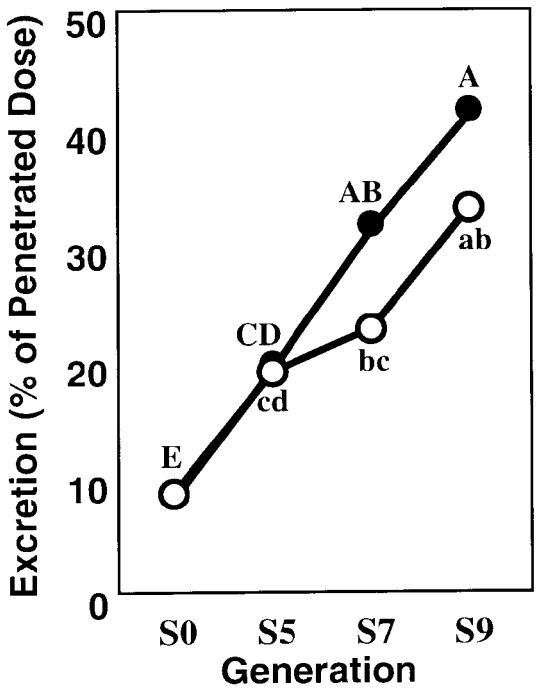

Figure 5: Levels of cypermethrin metabolites extracted from feces of adult *H. virescens*. Strains tested were selected as either larvae (closed circles) or adults (open circles).

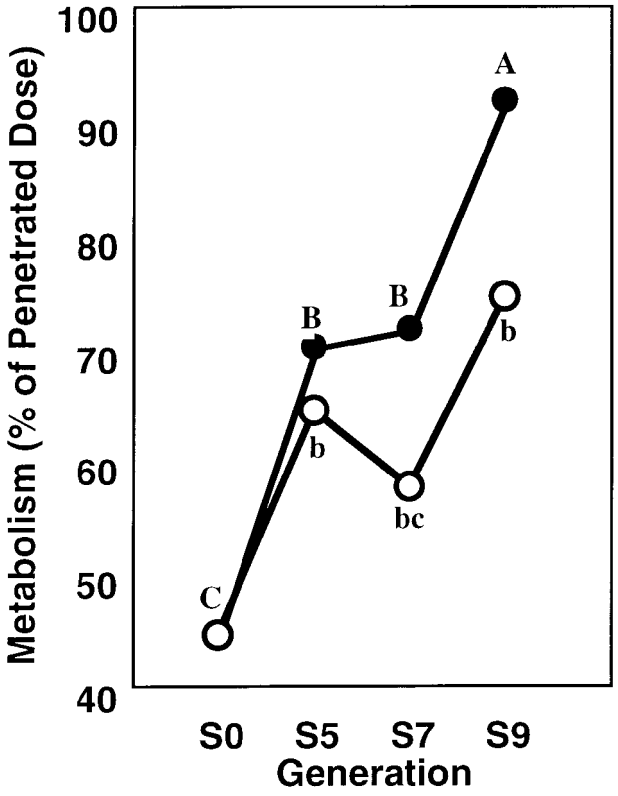

Figure 6: Levels of cypermethrin metabolites extracted from feces and carcasses of adult *H. virescens*. Strains tested were selected as either larvae (closed circles) or adults (open circles).