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INSECTICIDE RESISTANCE TESTING IN THE COTTON PINK BOLLWORM PECTINOPHORA GOSSYPIELLA

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ABSTRACT

LC_{50} values for third instar pink bollworm were obtained for laboratory susceptible and field strains (Palo Verde Valley, CA) for the following compounds: permethrin, chlorpyrifos, malathion, methomyl, chlordimeform, S,S,S-tributyl phosphorotrithioate (DEF), piperonyl butoxide (PBO), and *Bacillus thuringiensis* subsp. *kurstaki* toxin. Mortality after topical applications was recorded at 24 and 48 hrs. LC_{50} values were also obtained for selected insecticide/synergist combinations. Resistance ratios were calculated for each insecticide and insecticide/synergist combination. Synergistic ratios were calculated for each combination for field and laboratory populations. At the same time, determinations of total protein and measurements of esterase activity (as naphthyl acetate) were also obtained in effort to correlate esterase activity to the presence of resistance in the field strain. Significant resistance was found in the field to permethrin and methomyl. Permethrin also was found to have a high synergistic ratio suggesting a possible metabolic resistance component. The field strain was found to have significantly higher esterase activity when compared to the laboratory strain. Though higher total protein levels were found in the field strain, some unknown percentage of resistance (possibly metabolic) can be attributed to elevated esterase levels especially for resistance to permetnrin.

INTRODUCTION

The pink bollworm *Pectinophora gossypiella* (Saunders) attacks more than 70 plant species (Nobel 1969) and is a major pest of cotton in most parts of the world, including the U.S. western belt (Burrows *et al.*; Gordon Gordh, personal communication). In the desert growing areas of California and Arizona this species has caused serious economic losses to cotton since 1966 (Ashworth *et al.* and Anonymous 1984). For the past several years, pink bollworm control in infested areas of the U.S. cotton belt has relied on organophosphates and synthetic pyrethroid insecticides. Resistance of pink bollworm larvae to DDT was first reported in Mexico (Lawry and Tsao 1961). Recent studies in Arizona and California have shown an increase in resistance of pink bollworm larvae due to pressure from intensive applications of pyrethroids (Bariola and Lindgren 1984; Bariola 1985; Haynes *et al.* 1986).

We compared the response of third instar pink bollworm larvae (laboratory susceptible) strains and a field strain (Palo Verde Valley) to chlorpyifos, malathion, methomyl, permethrin and to *Bacillus thuringiensis* subsp. *kurstaki* toxin. We also tested permethrin for synergism with chlordimeform (CDF), and with the oxidase inhibitor piperonyl butoxide (PBO), as well as malathion for synergism with the esterase inhibitor S,S,S-tributyl phosphorotrithioate (DEF). CDF was also tested in combination with methomyl or chlorpyrifos. The esterases present in the two strains were compared by starch gel electrophoresis. Assays of esterase activity and total protein content determination were obtained. We compared laboratory and field strains as to esterase activity and discuss the relationship of esterase activity to resistance to permethrin.

MATERIALS AND METHODS

Insects

The susceptible strain (laboratory strain) of pink bollworm was provided by T.C. Baker, Univeristy of California, Riverside. The strain has been reared in the laboratory since 1978 without exposure to insecticide under a photoperiod of 14:10 (L:D) and temperature of 26±1 C° (Haynes *et al.* 1986).

Cotton bolls infested with pink bollworm larvae were collected from Palo Verde, California and were held in wire racks in the laboratory. Metal trays lined with paper towels were placed under the racks for collection of pupating larvae. These animals were subsequently placed indvidually in cups and held in an environmental chamber under the same conditions as the susceptible strain (Bartlett and Wolf 1985). All cultures were maintained on an artificial diet of casein, granulated sugar, Wesson's salts, Alphacel, wheat germ, vitamin diet mixture, KOH, formalin and acetic acid (Adkisson *et al.* 1960) and Vanderzant *et al.* 1962).

Chemicals

All chemicals used were of technical grade and were obtained from the sources indicated: permethrin 94.6% (FMC Corp., Princeton, N.J.); chlorpyrifos 98% (Dow Chemical Co., Midland, Mich.); malathion 91% (American Cyanamid, Princeton, N.J.); methomyl (DuPont, Baltimore, DE); chlordimeform (Ciba-Geigy, Greensboro, NC); DEF 80% (Mobay Corp., Kansas City, MI); PBO (MGK Corp., Minneapolis, MN; and *Bacillus thuringiensis* subsp. *kurstaki* toxin (FOIL)(Ecogen, Inc. Langhorne, PA.).

Bioassay

Serial dilutions of the chemicals were prepared in acetone, except for BT(k) which was suspended in water. The insecticides were tested on the susceptible and field strains by topical application of 1 µl drops to the thoracic region of third instar larvae (weighing ca. 11 mg), using an Arnold hand microapplicator (Burkhard Manufacturing Co., Ltd., Rickmansworth, England). Five to eight concentrations of each insecticide,

insecticide-synergist combination, BTK and controls (acetone only) were applied to groups of 10 larvae and were repeated on four different days. Tests on the field strain were conducted using F_1 and F_2 larvae. Each group of ten larvae were provided with 2 g of artifical diet, and were held in an environmental chamber at 26±1 C° and a photoperiod of 14:10 (L:D). Mortality was recorded after 24 and 48 hrs. The criterion of death was inability to react when prodded with the point of a needle.

Synergism study

Synergists were applied one hour prior to treatment with insecticide. Synergist sensitivity was established in preliminary tests, so that the dose chosen represented the maximum sublethal concentration (LD1) divided by 2. The doses at which three synergists were applied were as follows: PBO 20 $\mu g/\mu l$, DEF 20 $\mu g/\mu l$, and CDF 20 μg/larva.

Esterase

The electrophoretic mobility of esterases of pink bollworm was determined in third instar larvae by starch gel electrophoresis according to a modification of the procedure described by Pasteur *et al.* (1981). This technique reveals esterases that hydrolyze α and β-naphthyl acetate (NA) by staining the esterase hydrolysis product (naphthol) with Fast Garnet GBC (Sigma FO875).

Esterase Activity Determinations

Esterase activity was determined in whole pink bollworm third instar larvae which were homogenized in 100 μl of ice cold phosphate buffer (0.1 M, pH 6.5) containing 0.5% Triton X-100. Homogenate was diluted to bring esterase activity to within standard values for esterase (2-20 nanomoles). Larval homogenate (50 μl) was placed in a 96-well microtiter plate. Once loaded 100 μl of substrate solution: 0.5 ml of 60 mM α-napthyl acetate in absolute ethanol; 7 ml deionized water; 2-5 ml phosphate buffer; 0.01 ml of 2 mM 1,5 bis (4-allyl dimethyl ammonium phenyl) pentan-3-one-dibromide (Wellcome Research Laboratory, Research Triangle Park, NC), was added to

the plate and incubated for 30 min (25°C). Fast Garnet GBC (100 µl of 0.8 mg/ml) was added to each well to develop the product. Wells were read at 550 nm 10 min after addition of developing agent. Six individuals/microtiter plate with eight replications was used and twenty four individuals were used for each strain.

Protein Determination

A modification of the Bradford method (Brogdon 1984) was used with aliquots of homogenates diluted with deionized water. Samples (placed in microtiter plates) were held on ice and 200 µl of 1:4 solution (v/v) of Bradford reagent added to each well. Plates were held at room temperature for 10 min and absorbance read at 600 nm.

Data Analysis

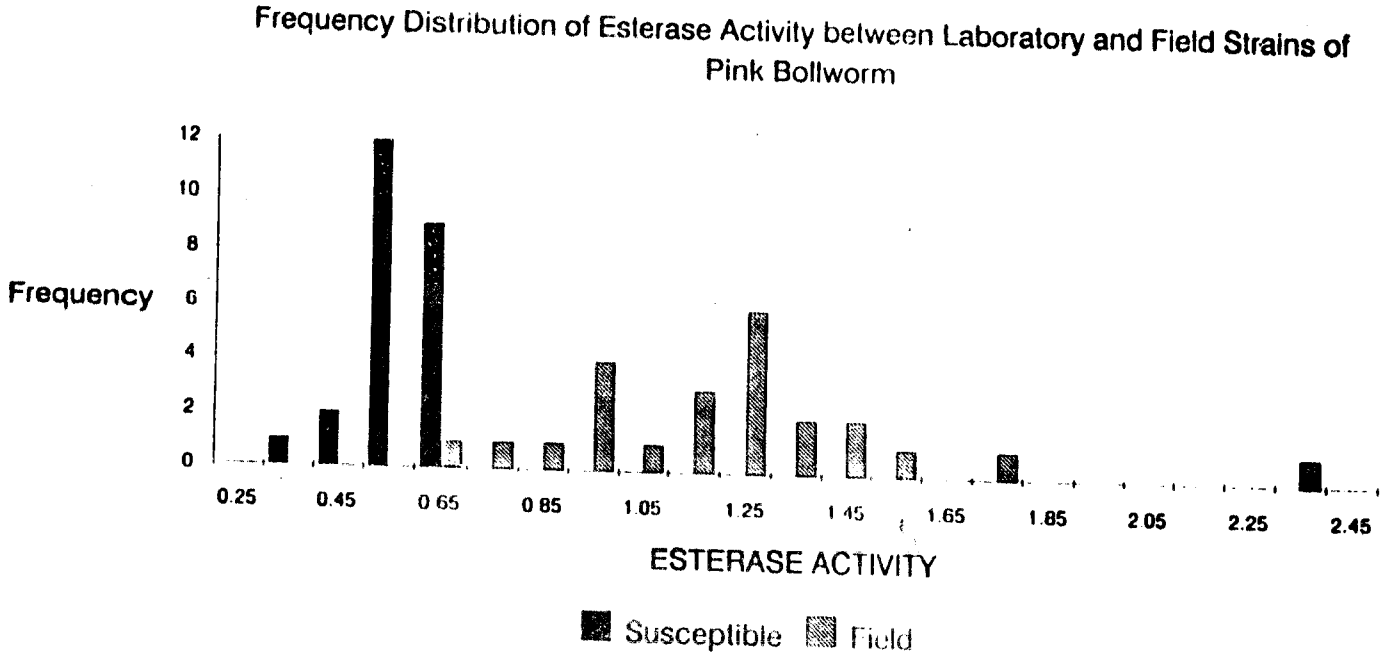
Results of the dosage mortality experiments were analyzed by probit analysis (Finney 1971). The following parameters were calculated: LD_{50}, resistance ratio, synergistic ratio, and relative synergism ratio. Absorbance was transformed to nmole α-naphthol with reference to an α-napthol standard curve for esterase activity determinatons, and to µmoles protein per larvae with refrence to gamma-globulin standard curve for protein deterination. Standard curves were run for all assays.

RESULTS AND DISCUSSION

The use of metabolic indicators as a measure of resistance has been used successfully in mosquitoes (Pasteur, et al, 1981; Ferrari and Georghiou, 1990) using titers of esterase activity as an index to resistance levels. These studies showed that high esterase activity in *Culex quinquefasciatus* was correlated with an increase in resistance to organophosphate insecticide. However, both studies indicated that the functional level of esterase activity is not a direct measure of resistance development in the animal. Levels of esterase activity may vary depending on the life stage of the animal and the genetic composition of the population being tested.

Using the two above studies as a guide, a modification of the esterase procedure was used in this study to determine if differences in laboratory and field pink bollworm

Fig. 1. Comparison of esterase activity between susceptible and field strains of pink bollworm. Esterase activity is measured in nanomoles a- naphthyl acetate hydrolyzed per minute per microgram protein. Note that the field strain has a wider range of activity suggesting a heterogeneous response to its host plant.



populations could be defined. Total protein concentrations on an individual level were found to be an order of magnitude higher than that of mosquitoes. This protein increase is probably a reflection of the size of the animal. This protein content should be reflected in a correspondingly increased esterase activity. Thus, pink bollworm with a correspondingly higher titer of esterase activity should show differences based on feeding preferences and rearing substrate. By adjusting for total protein, comparisons can be made between field and laboratory strains.

When esterase activity was compared between the field and laboratory strains of pink bollworm (Fig. 1), the field strain was found to have a significantly higher esterase activity (mean = field 1.23±0.37 nanomoles; susceptible 0.57±0.08 nanomoles). Field strain esterase activity was also found to be distributed over a larger range of values than the laboratory strain of pink bollworm. This larger distribution probably reflects a greater heterogeneity found in the field population.

Topical toxicity results are presented in Table 1. Resistance ratios (RR) within an insecticide were similar for 24 and 48 hr exposure times with RR values increasing slightly except for permethrin where the RR value droppped from 18.3 to 11 after 48 hrs exposure. Little resistance was shown for chlorpyrifos, malathion and BT(k) but the RR values for methomyl and permethrin indicated that resistance was present in the field population for these two compounds. Synergistic ratios (SR) were also higher for these two compounds. The SR was highest for permethrin with PBO indicating that there may be a significant metabolic factor leading to the development of resistance in the field population we tested.

In Fig. 2 we compare the esterase activity found in larvae from field and laboratory strains via starch gel electrophoresis. There was a visible difference with the field population having higher amounts of protein. Greater protein content in the field population may reflect a larval response to its host plant. When analyzing the esterase activity between the two populations, these values were also higher. By compensating the

Table 1. Topical toxicity of different insecticides and *Bacillus thuringiensis* (k) to susceptible and resistant third instar larvae of the pink bollworm *Pectinophora gossypiella*.

Compound	Exposure Time (h)	Strain	LC_{50}[a] (µg/ins)	95% C.I.	Slope (±SE)	RR[b]	SR[c]
Chlorpyrifos	24	S	01.5	(0.80-2.40)	1.01±0.14		
	24	R	02.0	(1.7-3.2)	0.93±0.17	1.3	
	48	S	00.5	(0.2-7.0)	0.95±0.13		
	48	R	01.0	(0.5-1.6)	1.01±0.15	2.0	
+CDF	24	R	01.7	(1.0-2.7)	1.03±0.13		1.2
	48	R	00.8	(0.5-0.14)	1.06±0.16		1.3
Malathion	24	S	32.8	(24.5-41.4)	1.55±0.20		
	24	R	47.6	(38.7-57.8)	1.93±0.25	1.5	
	48	S	24.0	(18.2-29.7)	1.81±0.20		
	48	R	32.7	(26.1-39.8)	1.91±0.24	1.36	
+DEF	24	R	38.5	(31.7-46.4)	2.03±0.24		1.24
	48	R	28.0	(23.0-33.2)	2.40±0.30		1.17
Methomyl	24	S	1.60	(0.9-2.6)	0.86±0.10		
	24	R	21.50	(17.6-26.2)	4.94±0.38	13.44	
	48	S	1.40	(0.2-0.8)	1.01±1.12		
	48	R	17.80	(14.1-21.5)	4.97±0.38	12.71	
+CDF	24	R	1.20	(1.03-1.6)	3.46±0.24		17.92
	48	R	0.95	(1.17-2.9)	2.82±0.19		18.84
Permethrin	24	S	00.40	(0.3-0.5)	1.84±0.10		
	24	R	7.30	(0.6-0.8)	3.73±0.34	18.25	
	48	S	0.60	(0.4-0.7)	3.61±0.35		
	48	R	6.60	(4.3-8.5)	3.07±0.31	11.00	24.3
+PBO	24	R	0.30	(0.2-0.3)	2.31±0.36		33.0
	48	R	0.20	(0.2-0.3)	2.49±.36		36.5
PBO+DEF	24	R	0.20	(0.2-0.3)	2.49±.36		44 0
	48	R	0.15	(0.1-0.2)	3.30±0.31		
Bt(k)	24	S	2.91	(2.25-3.66)	2.97(±0.48)		
	24	R	7.53	(2.75-18.90)	0.89(±0.34)	2.59	
	48	S	1.66	(1.08-2.26)	2.64(±0.67)		
	48	R	5.57	(4.15-7.32)	3.08(±0.61)	3.36	

[a]Each LC_{50} is based on four replicates; each replicate consisted of ten larvae for each insecticide concentration or untreated controls.

[b]Resistance ratio (LC_{50} for R strain/LC_{50} for S strain).

[c]Synergistic ratio (nonsynergized LC_{50}/synergized LC_{50}).

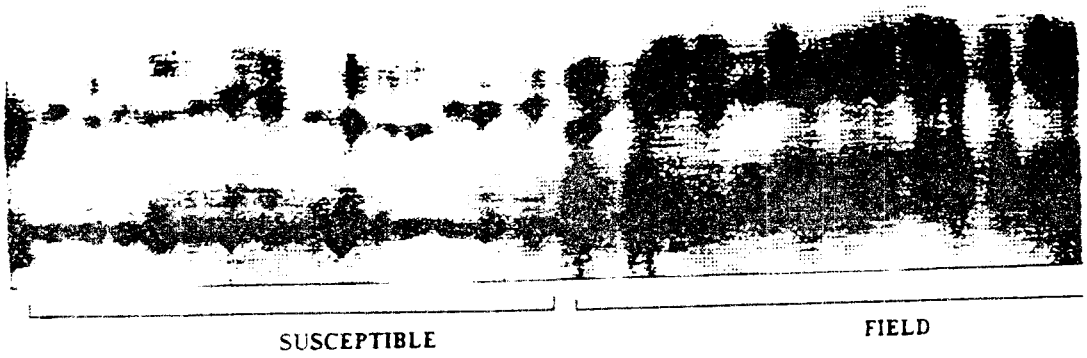


Fig. 2. Starch gel electrophoresis comparing esterase activities between field and laboratory susceptible strains of pink bollworm larvae. The field strain (on the right) shows a considerably greater amount of esterase activity for all larvae tested.

esterase readings by adjusting for total protein, true esterase activity measurements were obtained. The increased level of esterase activity in the larvae may be a response by the larvae to its environment but there is also the possibility that some of this elevated esterase activity is attributed to elevated resistance levels in the field population particularly for permethrin. Further study is required to determine what percent of esterase activity can be directly attributed to insecticide resistance.

Tests comparing field and laboratory strains of pink bollworm are a necessary element to understanding resistance problems. However, many tests of resistance lack the sensitivity that is required to determine if a low level of resistance is present within a specific population. Even when significant differences are noted in a test, there is still the problem of determining if that difference is meaningful in terms of resistance mechanisms. Many times the actual resistance mechanism is not known. It is important that more work be done in establishing sensitive tests for resistance and to implement these tests within the context of a resistance monitoring program.

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