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Study of Relationship between Pyrethroid Structure and Resistance in Cotton Bollworm *Helicoverpa armigera* (Hubner)

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Abstract

Resistance of insects to insecticides continues to be a serious barrier to successful management of insect pests. Field populations of *Helicoverpa armigera* have developed resistance to every class of insecticide over the past 40 years. Insects can develop resistance through reduced target site sensitivity, enhanced metabolism and decreased penetration. In the present study, the degree to which cross-resistance is conferred between Ops and pyrethroids by selection for enhanced esterase activities were examined. Resistance was highest to the insecticide used for selection In addition; pyrethroid resistance was higher to insecticides with 3-phenoxybenzyl alcohol/aldehyde, negative cross-resistance was not measured for either acephate or indoxacarb. In contrast, high levels of cross- resistance to indoxacarb existed in both OP-R (RR: 15.8) and PYR-R (RR: 5.57) strains, a low level of resistance to spinosyn A was measured in OP-R (RR: 2.19) and PYR-R (RR: 2.99) larvae. The present studies suggest that the chemical structure of an insecticide is an important determinant of metabolism and cross-resistance. The objective of this study was to investigate the relationship between pyrethroid structure and cross-resistance in *Helicoverpa armigera.*

1. Introduction

Resistance is defined as "*the ability of an insect population to survive a dose of poison that is lethal to the majority of individuals in a normal population of the same species*". Development of metabolic or target resistance to a particular insecticide may result in hypersensitivity (i.e., negative cross-resistance) to other insecticides that have not been previously applied. Mechanistically, negative cross-resistance may arise in a number of ways. First, enhanced activities of detoxifying enzymes accompanied with resistance to one insecticide may increase the bioactivation of another. The increased activities of cytochrome P450 monooxygenases were possibly responsible for observed negative cross-resistance to cypermethrin in a chlorpyrifos-selected strain of the German cockroach, *Blattella germanica* (Scharf et al., 1997) [1], to diazinon in the pyrethroid resistant horn fly, *Haematobia irritans* (Cilek et al., 1995) [2], to chlorpyrifos in dicofolselected two-spotted spider mites, *Tetranychus urticae* (Hatano et al., 1992) [3] and to chlorfenapyr in pyrethroid-resistant *Helicoverpa armigera* and *Haematobia irritans* (Sheppard and Joyce, 1998) [4]. Enhanced activities of esterases were associated with observed negative cross-resistance to proinsecticides in insecticideresistant peach-potato aphid, *Myzus persicae* (Hedley et al., 1998) [5]. In addition to increased metabolic activation, structural changes in insecticide target sites accompanied with resistance to an insecticide may become more sensitive to other insecticides that share the same target site, allosteric effects at target sites may result in negative cross-resistance between two types of insecticides that share the same target, but different sites.

The development of metabolic or target resistance to a particular insecticide may often result in high levels of resistance (i.e., cross-resistance) to other insecticides that have not been previously applied. For example, DDT and pyrethroids share the same target site on the voltage-sensitive sodium channels. Thus, the wide variation in susceptibility of *Helicoverpa armigera* to the pyrethroids before their widespread field applications is thought to result from cross-resistance associated with reduced sensitivity of this target site arising from the previous and extensive use of DDT (Davis et al., 1975; Brown et al., 1982; Leonard et al., 1988) [6,7,8]. Similarly, resistance due to reduced sensitivity of acetylcholinesterases in *Helicoverpa armigera* conferred cross-resistance among many OPs and carbamates (Brown and Bryson, 1992; Heckel et al., 1998) [9,10]. Similar metabolic mechanisms underlying resistance to

OPs, carbamates and pyrethroids in *Helicoverpa armigera* conferred cross-resistance among these three classes of insecticides (Campanhola and Plapp, 1987; Leonard et al., 1988) [11,8]. The cross-resistance to structurally divergent Bt toxins in *Helicoverpa armigera* was exhibited following selection with a *Bt* toxin (Cry1Ac) in the laboratory (Gould, 1992) [12] and is not accompanied by significant alterations in toxin binding. Generally, metabolic resistance is believed to confer a broader cross-resistance than target site insensitivity (Casida and Quistad, 1998) [13].

The spectrum of cross-resistance not only depends on the target sites and metabolic pathways of an insecticide, but also on the insect being selected and the chemical structure of the insecticide used for selection. Both natural pyrethrins and synthesized pyrethroids share the same target site (i.e., voltage sensitive sodium channels), but pyrethroid-resistant *M. domestica* had very high levels of resistance to synthesized pyrethroids (e.g., permethrin: 204-fold; phenothrin: 283-fold), but had only 7-fold resistance to natural pyrethrins (Katsuda, 1999) [14]. Similarly, permethrin-selected resistant strains of *Helicoverpa armigera* expressed varying degrees of cross-resistance to other pyrethoids (Brown et al., 1996) [7]. Moreover, the pyrethroid, fenfluthrin, was not resisted by a strain of *Helicoverpa armigera* that was selected for high levels (i.e., 58-fold) of resistance to cypermethrin (Shan et al., 1997) [15]. In India resistance to chlorpyriphos was highest (3.65-8.25 fold) (Nimbalkar et al., 2009) [16].

2. Materials and Methods

Study area

Larvae of Helicoverpa armigera were collected from the different districts of Maharashtra especially from Marathwada region i.e. Beed, Latur and Osmanabad District, during August to October 2009.

Insects

A susceptible strain of *Helicoverpa armigera* was established and has been reared in the laboratory since that time without exposure to insecticides. All larvae for resistance selection were fifth instars. Larvae from the strain were selected for five consecutive generations with topical applications of profenofos at doses corresponding to the LD_{60} for each generation (3.5, 4.6, 8.5, 13.6 and 23.8 μg/individual). After one year without selection, individual larvae were treated with 25 μg profenofos (an approximate LD_{20}), and the survivors (OP-R strain) were used for biochemical and toxicological assays. Similarly, larvae from the strain were selected for four consecutive generations with topical applications of cypermethrin at doses corresponding to the LD_{60} (0.22, 0.4, 0.5 and 0.6 μg/individual). After one generation without selection, larvae were treated with 1 μg of cypermethrin (6% mortality at 72 hours), and the next generation (PYR-R) was used for biochemical and biological assays.

Chemicals

Permethrin (94.6%), bifluthrin (96%), tefluthrin (97%), cypermethrin (96%) and acephate (97.6%) Indoxacarb (100%) and spinosyn A (100%) *trans*- Fenfluthrin was originally synthesized by Shan et al., (1997) [15] and re-crystallized before use.

Bioassays

Fifth instars (day 1), weighing 180±20 mg, were treated on the mid-thoracic dorsum with 1 μl aliquots of either profenofos or cypermethrin (in acetone) or with acetone alone (control). The dose-mortality relationship for each compound was assessed from at least five doses with 30 insects treated per dose. After treatment, larvae were maintained at 27°C, and mortality was recorded after 72 hours. The criterion for mortality was the lack of coordinated movement within 30 seconds after being prodded. Results were corrected for control mortality with Abbott's formula (Abbott, 1925) [17], then analyzed by Finney's method (Finney, 1971) [18]. Resistance ratios (RR) were calculated as LD50 of resistant strain / LD50 of susceptible strain. Values were considered statistically different if their 95%.

3. Results

Resistance to all insecticides tested (except for acephate) was present in both OP-R and PYR-R larvae (Table 1). Resistance to different insecticides varied depending on the strain used for assays. Resistance was highest to the insecticide used for selection (i.e., to profenofos in the OP-R strain and cypermethrin in the PYR-R strain). In addition, pyrethroid resistance was higher to insecticides with 3-phenoxybenzyl alcohol/aldehyde (e.g., cypermethrin and permethrin) than those with 3-phenyl 2-methylbenzyl alcohol (e.g., bifenthrin), and with fenfluorobenzyl (e.g., fenfluthrin) or 4-methyl tetrafluorobenzyl alcohol (e.g., tefluthrin). Moreover, negative cross-resistance was not measured for either acephate or indoxacarb. In contrast, high levels of cross- resistance to indoxacarb existed in both OP-R (RR: 15.8) and PYR-R (RR: 5.57) strains.

In addition, a low level of resistance to spinosyn A was measured in OP-R (RR: 2.19) and PYR-R (RR: 2.99) larvae.

Table 1: Susceptibility of larvae from the susceptible (IND- 4) and resistant (OP-R and PYR-R) strains of *Helicoverpa armigera*

Strain[a]	Pesticide	LD_{50} (95% FL[b])	Slope (SD[c])	Chi-square	RR[d]
IND-4	profenofos	2.90 (2.40-3.56)	2.63 (0.40)	1.38	
	cypermethrin	0.19 (0.16-0.24)	2.81 (0.39)	2.90	
	permethrin	0.14 (0.11-0.19)	1.91 (0.25)	0.93	
	tefluthrin	0.51 (0.42-0.69)	2.65 (0.52)	0.05	
	bifenthrin	0.05 (0.04-0.06)	3.12 (0.42)	3.08	
	trans-fenfuthrin	1.64 (1.22-2.12)	2.99 (0.40)	3.73	
	spinosyn A	1.84 (1.35-2.66)	1.66 (0.29)	0.49	
	indoxacarb	0.27 (0.22-0.33)	4.46 (0.64)	2.05	
	acephate	25.8 (22.3-29.4)	4.26 (0.61)	1.65	
OP-R	profenofos	52.3 (46.0-59.0)*	3.88 (0.57)	1.76	18.1
	indoxacarb	4.29 (3.18-5.87)*	1.62 (0.23)	1.11	15.8
	cypermethrin	2.46 (1.86-3.43)*	6.49 (0.88)	1.06	12.9
	permethrin	1.03 (0.54-1.75)*	2.05 (0.31)	3.99	7.20
	bifenthrin	0.17 (0.13-0.24)*	1.77 (0.34)	2.03	3.47
	tefluthrin	1.68 (1.38-2.01)*	3.00 (0.42)	2.38	3.30
	trans-fenfluthrin	4.30 (3.70-4.88)*	4.94 (0.64)	0.16	2.59
	acephate	69.1 (55.0-86.8)*	3.64 (0.53)	4.76	2.76
	spinosyn A	4.03 (2.85-5.49)*	1.52 (0.22)	0.64	2.19
PYR-R	cypermethrin	3.75 (3.16-4.33)*	3.34 (0.48)	1.56	19.6
	permethrin	5.59 (4.27-7.91)*	1.77 (0.28)	0.57	12.4
	bifenthrin	0.26 (0.18-0.41)*	2.97 (0.38)	8.40	5.10
	trans-fenfluthrin	4.83 (3.91-6.25)*	4.49 (0.65)	3.18	2.91
	tefluthrin	1.11 (0.96-1.31)*	1.77 (0.28)	6.75	2.18
	profenofos	13.2 (10.4-16.2)*	2.49 (0.39)	2.17	4.53
	spinosyn A	5.49 (3.79-7.93)*	1.30 (0.21)	0.19	2.99
	indoxacarb	1.51 (1.02-2.06)*	1.68 (0.29)	0.44	5.57
	acephate	39.4 (24.8-62.5)	5.09 (0.61)	9.48	1.53

[a]Strain IND-4 : insecticide-susceptible; OP-R: profenofos–selected; PYR-R: cypermethrin-selected
[b]FL= fiducial limits. *: Significantly different from the corresponding datum in the IND-4 strain
[c]SD=standard deviation
[d]RR=synergism ratio, defined as LD50 (without a synergist) / LD50 (with a synergist)

4. Discussion

Chemical structure, as it relates to metabolic stability, is a major determinant of crossresistance; however, the influence of structure on cross-resistance spectra is poorly understood. In OP-resistant *Lucilia cuprina*, esterases are primarily responsible for resistance (Campbell et al., 1998). Insects resistant to diazinon (a diethoxy ester) displayed 2-fold more resistance toward other diethoxy OPs than their dimethoxy analogs. Similarly, these strains did not show cross-resistance to either diisopropyl or OPs with chiral phosphorus atoms. In contrast, strains selected with malathion (a dimethoxy ester) displayed 2-5 times more resistance toward the dimethoxy OPs than their diethoxy analogs. Moreover, these strains showed only slight resistance (<3-fold) to either the diisopropyl or optically active OPs, including the diisopropyl analog of malathion.

Structure activity relationships, as they relate to cross-resistance to pyrethroids, have received limited attention. In the present study, a relationship between pyrethroid structure and resistance (**Table 1**) has been examined with profenofos- and cypermethrin-resistant *Helicoverpa armigera* in which esterases and oxidases were primarily responsible for resistance. Four type-I (non-cyano) pyrethroids (permethrin, bifenthrin, tefluthrin and *trans*-fenfluthrin) showed much lower resistance ratios than a type-II pyrethroid (cypermethrin) in both OP-R and PYR-R strains. Similar results were obtained previously with a different, cypermethrin-selected strain of Helicoverpa armigera (Shan et al., 1997) [15]. In previous studies, permethrin-selected Helicoverpa armigera expressed greater resistance to type-I (permethrin) than type-II (cypermethrin) pyrethroids (Jensen et al., 1984; Brown et al., 1996) [19,7]. These results suggest that the insecticide used for resistance selection has a major effect on the degree of cross-resistance to other insecticides. In addition, the variability seen in resistance among the pyrethroids tested in this study suggests that target site resistance is not a major mechanism of resistance in the PYR-R strain. Differences in resistance among four different type-I pyrethroids were present in both OP-R and PYR-R strains. The phenoxybenzyl moiety of cypermethrin and permethrin is a major site for enzymatic detoxication (Little et al., 1989; Lee et al., 1989) [20,21]. Tefluthrin and *trans*-fenfluthrin are structurally similar: both have fluorinated phenyl

rings, thus, a major site for detoxication by oxidases is no longer present. Resistance to these two insecticides was very similar (from 2.5- to 3.3-fold), but much lower than that to permethrin in both OP-R and PYR-R strains, suggesting that: 1) oxidases were involved in resistance in both resistant strains, which agreed with results from bioassays with TCPB; 2) a substitution of one chlorine atom in *trans*-fenfluthrin with a methyl group in tefluthrin did not change the degree of resistance in both OP-R and PYRR strains; 3) these two insecticides cannot be used as diagnostic compounds of esterases associated with pyrethroid resistance, but are useful for insects with oxidasemediated metabolic resistance. Moreover, the alcohol moiety of bifenthrin (i.e., 3-phenyl 2-methyl benzyl alcohol) probably subjects to some metabolisms. This was supported by higher resistance ratios of bifenthrin than those of tefluthrin and *trans*fenfluthrin, in the PYR-R strain.

The low level of resistance to non-phenoxybenzyl pyrethroids probably reflects the contribution of a penetration mechanism. Similar levels of resistance to spinosyn A in both OP-R and PYR-R strains (less than 3-fold) further suggest that a penetration resistance may be present in both resistant strains.

In summary, the spectrum of cross-resistance to pyrethroids in both profenofos- and cypermethrin-resistant strains was somewhat specific and was primarily dependent on the insecticide used for resistance selection. However, resistance to tefluthrin and *trans*fenfluthrin, in which some metabolic sites for detoxifying enzymes (e.g., oxidases) are blocked, may develop more slowly than those (e.g., permethrin, bifenthrin) in which sites for detoxication are present. These results suggest that simple bioassays with such compounds may be used to detect metabolic resistance in insects. In addition, similar modification in alcohol moiety of existing pyrethroids may result in insecticides that will be active against cotton bollworm expressing metabolic resistance.

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