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PY-R STRAIN OF TOBACCO BUDWORM (LEPIDOPTERA: NOCTUIDAE): MULTIPLE AND CROSS RESISTANCE AND SELECTION FOR RESISTANCE AND REVERSION TO SUSCEPTIBILITY TO PYRETHROID AND OTHER INSECTICIDE CLASSES

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Abstract

Progeny of multiple pairs of brother sister moths of a strain of the tobacco budworm, *Heliothis virescens* (F.) (TBW), ,designated PY-R, were treated for 14 generations with topical applications of 14 insecticides to determine LD50s. The strain was resistant to cyclopropane pyrethroids prior to the initiation of this test. Resistance was maintained for all the cyclopropane pyrethroids including bifenthrin (Capture), cyfluthrin (Baythroid), cypermethrin (Ammo), deltamethrin (Decis), permethrin (Pounce) and tralomethrin (Scout) during the generations of selection. Resistance to endosulfan (Thiodan), methyl parathion(Methyl Parathion) and sulprofos (Bolstar) was also determined. Resistance and susceptibility for the pyrethroids and macrolactones, i.e. abamectin (Zephyr) and emamectin benzoate (Denim), are based on a resistance threshold of LD50 >0.2 µg/larva. Resistance to anticholinesterase insecticides and endosulfan is based on a resistance threshold of an LD50 of >20 µg/larva. PY-R was susceptible to emamectin benzoate (Denim), methomyl (Lannate) and profenofos (Curacron) in one, one and three generations, respectively. The strain was susceptible to esfenvalerate (Asana), a non-cyclopropane pyrethroid, in generation five, when it was first tested, resistant in generations nine through 13 and susceptible again in generation 14. Multiple and cross resistance was evident by this strain. Inheritance patterns have to be polygenic.

Introduction

A strain of the tobacco budworm, selected for resistance to cyclopropane pyrethroids, was maintained by Bob Blenk, of ICI, at Goldsboro, NC, and Walnut Creek, CA, from 1978 to the 1990s (Firko and King 1991). Blenk achieved resistance by this strain by (1) selection pressure with cyclopropane pyrethroids to each larva each generation and (2) addition of field collected strains of TBW, considered to be resistant to cyclopropane pyrethroids, to this resistant strain designated PY-R. This strain of the TBW has been used extensively to evaluate various aspects of resistance to cyclopropane pyrethroids in the United Kingdom and the United States of America (USA) in the 1980s and early 1990s. The strain was made available by Bob Blenk for research projects.

The strain was first used to study the inheritance of resistance to permethrin (Payne et al. 1990) and to increase resistance of a male lethal mutant strain to cypermethrin (Firko and King 1990 and1991). The strain was also used to determine time for toxicity and criteria for death by cypermethrin (Firko and Hayes 1991). and toxicity of cypermethrin and anticholinesterase insecticides to neonate, third instar larvae and adults (Campanhola and Plapp 1989ab). The strain was used to evaluate the role of synergists for toxicity with cypermethrin (Campanhola and Plapp 1989ab, McCaffery et al. 1991 and Brown et al. 1996) and chlorfenapyr against this insect (Pimprale, et al. 1997). The strain was also used in other inheritance of resistance studies to permethrin (Heckel et al. 1992), Cypermethrin was tested for mode of action and toxicity against this strain (Schriebner and Knowles 1989, McCaffery et al. 1991, Taylor, et al. 1993 and Lee et al. 1999).

Selection for resistance and reversion to susceptibility and cross and multiple resistance to 14 insecticides was determined for multiple pairs of brothers sisters of the PY-R strain for 14 generations. Focus was to determine the toxicity and cross resistance of cypermethrin, a cyclopropane pyrethroid vs. esfenvalerate, a non-cyclopropane pyrethroid. Resistance to a non-cyclopropane pyrethroid has not been determined. In addition, cross and multiple resistance to five other cyclopropane pyrethroids, three anticholinesterase, two macrolactone insecticides and one cyclodiene insecticide were also determined. Two resistance thresholds were determined. One was shown for pyrethroids and macrolactons and another for insecticides of the remaining classes.

Materials and Methods

Technical abamectin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, emamectin benzoate, endosulfan, methomyl, methyl parathion, permethrin, profenofos, sulprofos and tralomethrin were obtained from their manufacturers. The five classes of insecticides tested were carbamate, cyclodiene, macrolactones, organophosphorus and pyrethroids.

The TBW strain was obtained from ICI, Walnut Creek , CA, by M. J. Firko, USDA-ARS, at Weslaco, TX, in October, 1988. I obtained larvae from M. J. Firko in June, 1989, and reared them to pupation. In July, moths eclosed, and were mated as multiple pairs of brother sisters and allowed to oviposit. Eclosed larvae were maintained on 10 to 15 ml artificial diet and treated as generation one. I identified the strain as PY-R (it was also called PYR-R, ICI. Peg-87 and Pyrethroid-R by different authors). Strain included 10 different strains which were also resistant to pyrethroids. They were collected from cotton fields in different states in the USA prior to October, 1988 (Firko and King 1991). The PY-R strain (also called ICI) was resistant to cypermethrin and permethrin for several generations, but then the strain was lost (Campanhola and Plapp 1989b). They obtained a second sample of the strain which was used to continue their studies.

From July, 1989, to October, 1990, larvae were reared to pupation on standard artificial diet at 27± °C, 60 to 80% RH and 1:1 ratio of light: : dark (12 : 12 h) in 30 ml plastic cups with cardboard caps. Larvae were counted in each generation as an indication of population sizes. In each generation 5 to 30 moths, as multiple pairs of brothers sisters, were placed in a 3.78L cardboard container for their 4 to 19 d lifetime. Cloth covers and hangers provided oviposition sites in the containers and were changed daily and held in 12 oz plastic cups sealed with plastic lids. Each additional 30 moths available each generation were placed in another 3.78 L container.

Upon eclosion neonate larvae were placed singly on diet in cups and held for treating. Third-instar larvae were treated with 6, eight or 16 doses of each insecticide in one microliter of nanograde acetone using a micro-applicator (ISCO, Inc., Lincoln, NE) when they were three to seven d and weighed 22 ± 6 (16 to 28) mgs. Larvae of the different generations grew at different rates so 4 to 89 larvae/dose/d/insecticide were treated in each replicate. Doses, as µg/larva, were 0.31 to 10 for abamectin, 0.0775 to 10 for bifenthrin, cyfluthrin, deltamethrin and tralomethrin, 1.56 to 400 for cypermethrin and permethrin, 0.006 to 200 for esfenvalerate, 0.39 to 50 for endosulfan, methomyl and sulprofos, 0.00095 to 0.25 for emamectin benzoate, 0.39 to 100 for methyl parathion and 0.195 to 25 for profenofos. All doses were diluted 50% from the next greatest dose. Mortalities for all insecticides were taken after 48 h. All available larvae were treated in one to 10 replicates with each insecticide each generation. A replicate for each insecticide is the number of larvae treated on one d.

The lowest dose of cypermethrin (1.56 µg/larva) and profenofos (0.195 µg/larva) was used to determine natural mortalities. No untreated larvae were used to determine natural mortalities in any generation.
This was done so that all larvae could be used in the selection regime every generation. Untreated larvae cannot be incorporated into the selection regime.

LD50s and their 95% confidence interval (CI) as µg/larva and slope ± standard error (SE) were determined by PC probit analysis of SAS (1988). The LD50s with overlapping CIs were equal and not significantly different. If the ratio of the slope/SE of slope was <1.96 (t_{∞} =1.96 at $P < 0.05$) the regression was non-significant and did not differ from zero. Non-significant regressions were considered to be resistant because (1) mortalities exceeded the threshold for that insecticide or (2) mortalities were <50% at indicated dose. One non-significant regression was susceptible because mortalities were >50% and the dose was less than threshold. Slopes of these regressions for each insecticide describe a response by the strain. Slopes were considered to be flat when they were <1.0. LD50s are ranked from greatest to lowest each generation.

An LD50 >0.2 µg/larva was established as a resistant threshold for cyclopropane, non-cyclopropane pyrethroids and the macrolactones (Martinez-Carrillo and Wolfenbarger 2004a). An LD50 >20.0 µg/larva was established for the anticholinesterase insecticides and the cyclodiene insecticide against this strain of TBW (Martinez-Carrillo and Wolfenbarger 2004b). The threshold was used to separate resistance from susceptibility for indicated insecticide.

Cypermethrin and esfenvalerate were tested in 10 and eight of the 14 generations, respectively. Profenofos, cyfluthrin and permethrin were tested in two generations. Abamectin, bifenthrin, deltamethrin, endosulfan, emamectin benzoate, methomyl, methyl parathion, sulprofos and tralomethrin were tested in a single generation.

Results and Discussion

The LD50 of the resistance threshold for all cyclopropane pyrethroids was >0.2 µg/larva in all generations of this strain (Table 1). Results indicate cross resistance for all these pyrethroids. The greatest LD50 (8,633.61 µg/larva) was determined for cypermethrin in generation one. No LD50 of any field collected strain showed an LD50 equal to or greater than this value. LD50s for bifenthrin, cyfluthrin, cypermethrin, deltamethrin, permethrin and tralomethrin ranged from 15.48 to 8,633.61 µg/larva, a 558 fold difference in generations four, five and nine through 13.

Non-significant regressions of cyclopropane pyrethroids are as follows. in generations two, six, seven and 13, mortalities of cypermethrin, at 800 µg/larva, the maximum dose tested, with slopes ± SE and the number of larvae treated, respectively, were 13% (0.17 ± 0.13) 325, 29% (-0.44 ± 0.3) 116, 25% (0.15 ± 0.33) 143. and 26% (-0.18 ± 0.12) 208 larvae, respectively. In generations three and six cyfluthrin, deltamethrin, permethrin and tralomethrin, at 10, 20, 800 and 10 µg/larva, the maximum dose tested, slopes ± SE and the number larvae tested were 11% (0.17 ± 0.19), 257, 0% (-0.01 ± 0.25), 225, 17% (0.16 ± 0.16) 577 and 10 % (0.028 ± 0.48) 165 larvae, respectively. Slopes and their SE were extremely variable. All these regressions indicate resistance.

LD50s of anticholinesterase insecticides, endosulfan and the macrolactones were also tested against third-instar larvae of this PY-R strain. The strain was susceptible to methomyl and profenofos, but was resistant to methyl parathion, sulprofos and endosulfan (Table 1). Mortality of endosulfan at 25 µg/larva was 0% for 103 larvae. Dose exceeded resistance threshold or mortality was <30%. The strain was susceptible to emamectin benzoate, but was resistant to abamectin based on the same resistance threshold used for the pyrethroids (Table 1). In generation four the LD50 for emamectin benzoate was 20,379 fold less than for by cyfluthrin.

In lieu of an untreated check the lowest dose of cypermethrin and profenofos killed 3% to 8% of the larvae treated in generations 1, 3, 4, 5, 8. 9, 11 and 13 to correct for mortalities, respectively. Natural mortalities of untreated larvae are deemed to be <10%.

LD50s of 6.54 and 10.96 µg cypermethrin/larva, in two generations, were determined for third instar larvae of PY-R (Schriebner and Knowles 1989 called it ICI). The lowest LD50 for cypermethrin was significantly greater (Table 1) than the LD50s shown by Schriebner and Knowles (1990).

This is the first report on cross resistance by the PY-R strain to seven cyclopropane pyrethroids and the non-cyclopropane pyrethroid. Cross resistance was shown to permethrin and deltamethrin in generation three, bifenthrin and cypermethrin in generation five, cyfluthrin, cypermethrin and tralomethrin in generation six, cypermethrin and esfenvalerate in generations eight, nine and 11 and cypermethrin, esfenvalerate and permethrin in generation 13 (Table 1). LD50s either exceeded >0.2 µg/larva or mortalities were <50% at >0.2 µg/larva.

Multiple resistance was shown in generation three to deltamethrin and permethrin vs. methomyl and in generation four to cyfluthrin, sulprofos and endosulfan vs. profenofos and cyfluthrin, sulprofos and endosulfan vs. emamectin benzoate (Table 1). Mechanisms for multiple resistance by this strain have to be involved.

Slopes >3.44 are indicative of homozygosity of regressions by this PY-R strain of TBW (Wolfenbarger and Bartlett 2003). None of the insecticides from generations one through 14 for PY-R indicated homozygosity of slopes. For 31 significant and non-significant regression of all insecticides 32%, 42%, 13% and 3% were determined for slopes of <0.5, 0.51 to 1.0, 1.1 to 1.5 and >1.51, respectively. Most (74%) of the slopes were flat (<1.0). Slopes of 0.51-1.0, 1.1-2.0 and >2.0 for the anticholinesterase insecticides were 20%, 60% and 20%, respectively. Slopes of <0.5, 0.51-1.0 and 1.1- 2.0 for cyclopropane and non-cyclopropane pyrethroids were 54%, 38%, and 8%, respectively; 92% of the slopes were flat (<1.0) (Table 1). A slope of 0.65 was determined for the same strain when cypermethrin was topically applied (Schriebner and Knowles 1989). Slopes of the macrolactones, i.e. abamectin, 0.61 and emamectin benzoate, 0.56, were flat and thus they were like the pyrethroids.

A clear example of selection for resistance and subsequent reversion to susceptibility is shown to the non-cyclopropane esfenvalerate against this PY-R strain (Table 1). In generation five the LD50 was <0.1 µg/larva. This LD50 indicated the only susceptibility to a non-cyclopropane pyrethroid. In generation nine the LD50 was 226.48 µg/larva, then fell to <6 µg/larva in generations 10 through 13, respectively. In generation 14 the strain was susceptible to esfenvalerate because 50% of the 108 larvae were killed with 0.006 µg/larva with slope ± SE as 0.63 ± 0.36. This is the only result indicating susceptibility with a non-significant regression.

Then number of larvae of brother-sister multiple pairings treated in generations one through 14 were 527, 325, 1,973, 2,050, 704, 538, 143, 479, 258, 954, 1,182, 1,061, 461 and 108, respectively. Larval populations each generation ranged from 108 to 2,050, a 19 fold difference. Peak populations are shown in generations 3-4 and 10-12 Valleys are shown in generations 7 and 14.

McCutchen et al. (1989) stated that there was a cost for resistance by multiple pairs of PY-R strain because there was a decrease in reproductive success. This was not shown with the population used here (Table 1). The strain was not lost in any generation.

Table 1. Toxicity of selected insecticides of brother-sister multiple matings of PY-R strain of tobacco budworm. 1989-1990.

Insecticide	Number Larvae Treated	Slope ± SE	LD50 (µg/larva)	95% CI (low-high)
		Generation 1 July, 1989		
Cypermethrin	527	0.62 ± 0.2	8,633.61	2,532.61-1.71 x 10 5
		Generation 3 September		
Methyl parathion	400	1.02 ± 0.24	44.67	21.83-194.52
Methomyl	455	0.71 ± 0.092	9.11	6.16-14.31
Abamectin	316	0.61 ± 0.21	4.03	1.34-433.26
		Generation 4 October		
Cyfluthrin	460	0.37 ± 0.1	550.23	51.91-8.72 x 10 5
Sulprofos	518	0.71 ± 0.15	31.21	15.39-116.51
Profenofos	389	2.13 ± 0.52	1.83	1.17-2.79
Emamectin benzoate	583	0.56 ± 0.13	0.027	0.0033-0.08
		Generation 5 November		
Cypermethrin	143	0.42 ± 0.22	2,567.0	
Bifenthrin	306	0.61 0.31	1,314.0	67.61-3.55 x 10 37
Esfenvalerate	255	0.52 ± 0.16	0.091	0.00018-0.45
		Generation 8 February, 1990		
Cypermethrin	297	0.78 ± 0.38	2,962.0	
Esfenvalerate	182	1.38 ± 0.23	0.59	0.4-0.83
		Generation 9 March		
Cypermethrin	73	1.12 ± 0.45	592.56	315.81-1,3688.0
Esfenvalerate	185	0.49 ± 0.2	226.48	23.94-3.8 x 10 72
		Generation 10 April		
Esfenvalerate	954	0.2 ± 0.055	5.87	2.2-18.92
		Generation 11		

		May		
Cypermethrin	90	0.58 ± 0.26	89.39	30.57-5.1x10 5
Esfenvalerate	467	0.54 ± 0.19	1.78	0.0038-5.87
Profenofos	625	1.29 ± 0.13	0.37	0.24-0.51
		Generation 12		
		July		
Esfenvalerate	1061	0.67 ± 0.066	0.43	0.25-0.64
		Generation 13		
		August		
Permethrin	90	0.92 ± 0.24	15.48	5.58-31.2
Esfenvalerate	163	0.5 ± 0.11	5.01	1.76-32.76

It is unknown what accounts for these differences in toxicity by the same strain of this insect. There may be a different mode of action for the third instar larvae which causes the toxicity of the non-cyclopropane esfenvalerate and the cyclopropane pyrethroids, i.e. bifenthrin, cyfluthrin, cypermethrin, deltamethrin, permethrin and tralomethrin (Table 1). It may mean that it is easier to select for a mechanism of resistance to the cyclopropane pyrethroids than to the non-cyclopropane pyrethroid.

Discussion

Mechanisms of resistance of the PY-R strain involve a delayed penetration by the pyrethroid into the larva, increased metabolic factors in different stages and nerve insensitivity (McCaffery, et al. 1991, Lee et al. 1989, Little et al. 1989 and Walker et al. 1990). The importance of the monooxygenase metabolic factor was emphasized because there was rapid oxidative metabolism of cypermethrin by NADP-dependent microsomes (Walker et al. 1990). They also determined slow esterase hydrolytic activity in strains of two supernatant species and microsomes. Larvae of PY-R strains treated with cypermethrin died at a slower rate compared to a susceptible strain [Firko and Hayes 1991).

Nerve insensitivity as a major mechanism of pyrethroids against this strain is advanced by McCaffery et al. (1989a), Gladwell et al. (1990), McCaffery et al. (1991) and Heckel et al. (1992). Results on nerve insensitivity of the PY-R strain when 10^{7} molar concentration of cypermethrin showed only 33% of the larvae displayed neuronal hyperactivity, but 28% failed to respond at all at this concentration [Gladwell et al. 1990). All susceptible insects displayed neuronal hyperactivity at this concentration.

Physiological and genetic studies have suggested that a modified sodium channel in this strain of TBW may be responsible for resistance to permethrin [Heckel et al. 1992). Molecular variation at a locus may be linked to resistance to permethrin. Heckel described a polymerase chain reaction methodology to determine these results. Do these same results also apply to bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, *lambda* cyhalothrin (Karate), tralomethrin and zeta cypermethrin (Forte) against this strain? This remains to be determined

Factors for resistance by third-instar PY-R larvae probably are regulated by major and minor genes.. Larvae with a combination of all mechanisms probably possess high levels of resistance to cypermethrin and permethrin throughout their larval life. Cross resistance is then assumed for bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, *lambda* cyhalothrin, permethrin, tralomethrin, and zeta cypermethrin.

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