Original Article

Cross Resistance and Synergism in Fenvalerate-Resistant Diamondback Moth, *Plutella xylostella* (L.)*

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Two fenvalerate-selected strains (KAR₈₄-FR and OKR₈₄-FR) of the diamondback moth, *Plutella xylostella* (L.) resistant to fenvalerate (racemic) >500-fold showed resistance to fenvalerate A (S-isomer) and fenvalerate B (R-isomer) as well. Studies on cross resistance revealed that the KAR₈₄-FR strain was low in cross resistance to phenthoate, prothiophos, cyanophos and methomyl, and that the OKR₈₄-FR strain was particularly low in cross resistance to these insecticides. Both fenvalerate-resistant strains showed a slight tolerance to acephate and dichlorvos but no cross resistance to cartap. Synergism of piperonyl butoxide (PB) and triphenyl phosphate (TPP) with fenvalerate A was high against both resistant strains at synergist ratios (SR) of LD₅₀ >17. Higher synergisms of TPP with fenvalerate A at LD₅₀ levels were observed in the susceptible strains (KAR₈₄ and OKR₈₄) than in the resistant strains, while higher synergisms at LD₉₅ levels were shown in the resistant strains than in the susceptible strains. On the other hand, synergisms of sesamin, sesamol, episamin and S-421 with fenvalerate A were low in the KAR₈₄-FR strain, while apparently high in the OKR₈₄-FR strain.

INTRODUCTION

It is well known that the diamondback moth, *Plutella xylostella* (L.) has developed resistance to more than 46 insecticides, including chlorinated hydrocarbons, organophosphates, carbamates and especially some newly-introduced pyrethroids, within a short period.¹⁾ In Japan, after fenvalerate was introduced for agricultural pests control in 1983, diamondback moths in Kagoshima and Okinawa Prefectures were found to have developed resistance to fenvalerate in 1984.^{2,3)}

Synergists can be used as a chemical countermeasure for insecticide resistance.⁴⁾ In the tests on pyrethroid-resistant strains of diamondback moths in Taiwan, Liu *et al.*,^{5,6)} and Chen & Sun⁷⁾ have reported synergism of certain pyrethroids with piperonyl butoxide (PB), an oxidase inhibitor. The degree of synergism observed is as high as 30-fold, suggesting that it may help improve pyrethroid efficacy.

We have obtained two fenvalerate-resistant strains of diamondback moths which had been collected in fields and selected with fenvalerate (racemic) in a laboratory.⁸⁾ Our present study is aimed to investigate the effect of synergists on the toxicity of fenvalerate A, (R, S) α -cyano-3-phenoxybenzyl (S)-2-(4-chlorophenyl)isovalerate against the two fenvalerate-resistant strains. The degrees of cross resistance of both resistant strains to various insecticides are also investigated.

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MATERIALS AND METHODS

1. Insect Strains and Maintenance

Two fenvalerate-resistant strains (KAR₈₄-FR and OKR₈₄-FR) of diamondback moths were selected with fenvalerate (racemic) from the strains (KAR₈₄ and OKR₈₄) which had been collected in fields in Kagoshima and Okinawa Prefectures in 1984.⁸⁾ After the last selection, both KAR₈₄-FR and OKR₈₄-FR strains showed >500-fold resistance to fenvalerate (racemic) (Table 1). Cross resistance of the selected strains to various insecticides was studied by using the above strains within three generations after the last selection. Then they were pressured with fenvalerate (racemic) (5000 and 2500 ppm, respectively) at every three generations until used for synergist experiments. The whole synergist experiments were conducted on the same generation of the insects. Insects were mass-reared and maintained under constant laboratory conditions (25°C, 16 hr light and 8 hr dark) as described previously.⁹⁾

2. Chemicals

The chemicals used were fenvalerate, (*R, S*)-

α -cyano-3-phenoxybenzyl (*R, S*)-2-(4-chlorophenyl)isovalerate (racemic, a mixture of fenvalerate A (*S*-isomer) and fenvalerate B (*R*-isomer)) (97.6% purity); fenvalerate A, (*R, S*)- α -cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-isovalerate (100%); fenvalerate B, (*R, S*)- α -cyano-3-phenoxybenzyl (*R*)-2-(4-chlorophenyl)-isovalerate (98.2%); dichlorvos (98%); phenothoate (95%); prothiophos (89%); cyanophos (95%); acephate (97.9%); methomyl (100%); cartap (97.7%); PB, piperonyl butoxide (100%); TPP, triphenyl phosphate (95%); sesamin (90%); sesamol (98%); episesamin (90%) and S-421, octachlorodipropyl ether (98%). All insecticides and synergists were dissolved in acetone and refrigerated at 4°C as previously described.⁹⁾

3. Toxicity Tests

To obtain LD₅₀ and LD₉₅ of synergized and unsynergized insecticides, third instar larvae (2.55 mg) were topically treated with 0.52 μ l of each chemical solution as previously mentioned.⁹⁾ Each chemical was applied to a group of 10 larvae at seven to nine doses with three replications. The effect was deter-

Table 1 Susceptibility of four strains of diamondback moths to fenvalerate (racemic), fenvalerate A and fenvalerate B at 24 hr after topical application.

Insecticide	Strain	SRE ^{a)}	LD ₅₀ (μ g/g)	RF of LD ₅₀ ^{b)}	LD ₉₅ (μ g/g)	RF of LD ₉₅ ^{c)}
Fenvalerate ^{e)}	KAR ₈₄ -FR	0.67	3740	3500	(957,000) ^{d)}	125,000
	KAR ₈₄	2.45	1.07	1.0	7.64	1.0
	OKR ₈₄ -FR	0.93	1020	580	(59,700)	7960
	OKR ₈₄	2.26	1.76	1.0	7.50	1.0
Fenvalerate A	KAR ₈₄ -FR	0.61	3330	5840	(192,000)	53,000
	KAR ₈₄	2.04	0.57	1.0	3.62	1.0
	OKR ₈₄ -FR	0.92	877	1620	(53,500)	16,200
	OKR ₈₄	2.10	0.54	1.0	3.30	1.0
Fenvalerate B	KAR ₈₄ -FR	*** ^{f)}	***	***	***	***
	KAR ₈₄	1.43	953	1.0	(14,300)	1.0
	OKR ₈₄ -FR	***	***	***	***	***
	OKR ₈₄	1.38	1510	1.0	(23,300)	1.0

a) Slope of regression equation.

b) Resistance factor of LD₅₀=LD₅₀ of selected strain/LD₅₀ of nonselected strain.

c) Resistance factor of LD₉₅=LD₉₅ of selected strain/LD₉₅ of nonselected strain.

d) Values in parenthesis are extrapolated.

e) Data of fenvalerate are from Noppun *et al.*⁸⁾ (the LD₅₀ of KAR₈₄-FR and OKR₈₄-FR strains before selection are 1.19 and 1.51 μ g/g, respectively).

f) Data not obtainable.

mined after simultaneous treatment with a mixture of fenvalerate A and a synergist (1:1 by weight). The preliminary study indicated that even at the highest concentration of a synergist itself did not kill larvae.

Following treatments, the larvae were maintained in a controlled chamber (25°C, 16L and 8D) as mentioned previously.⁹⁾ Mortality data at 24 hr were used to compute dose-response data according to the Probit analysis¹⁰⁾ by a microcomputer (NEC PC-9801F, NEC Co. Ltd., Tokyo). Cross resistance was expressed by the values of resistance factor (RF) of LD₅₀ and LD₉₅.⁹⁾ Synergistic effect was evaluated based on the synergist ratios (SR) of LD₅₀ and LD₉₅.⁵⁾

RESULTS AND DISCUSSION

1. Susceptibility of Fenvalerate-resistant Strains to Fenvalerate A and Fenvalerate B

Fenvalerate (racemic)-selected strains (KAR₈₄-FR and OKR₈₄-FR) of diamondback moths showed resistance to fenvalerate A as well (Table 1). Resistance levels of the KAR₈₄-FR and OKR₈₄-FR to fenvalerate A were very high and almost the same as to fenvalerate (racemic). To evaluate LD₅₀ and LD₉₅ levels of fenvalerate B (a weak toxic isomer¹¹⁾) to the resistant strains was impossible, because even at the highest concentration (15,300 µg/g) it did not kill them. To the susceptible strains, the LD₅₀ and LD₉₅ of fenvalerate (racemic) were twice as large as those of fenvalerate A. If fenvalerate A and fenvalerate B in fenvalerate (racemic) are fifty-fifty, the toxicity of fenvalerate (racemic) is totally derived from fenvalerate A. Nakayama *et al.*¹¹⁾ have reported that synthetic pyrethroids with an α -cyano-3-phenoxybenzyl alcohol moiety, which have an *S* configuration at the acid moiety, have a higher potency to insects, acari and mammals than those having an *R* configuration. The relative potency of an *S*-isomer to an *R*-isomer is 70–100 against the house fly, *Musca domestica*, but only 5–10 against the pale house mosquito, *Culex pallens*. Our study found that fenvalerate A (*S*-isomer) was 1600 times more potent than fenvalerate B (*R*-isomer) against the susceptible strains. This tendency seemed also apparent in the resistant strains, even

though the LD₅₀ levels of fenvalerate B could not be determined for the resistant strains.

2. Cross Resistance of the Fenvalerate-resistant Strains to Various Insecticides

The KAR₈₄-FR strain showed cross resistance to phenthoate, prothiophos, cyanophos and methomyl. The resistance factor (RF) values of LD₅₀ of these insecticides were 13.7, 24.2, 53.4 and 37.2, respectively (Table 2). However, these RF values of LD₅₀ were smaller than the RF values of LD₅₀ of fenvalerate (racemic) (>580) (Table 1). Compared with the RF values of LD₉₅ of fenvalerate, the RF values of LD₉₅ of these insecticides were extremely small. To these insecticides, the OKR₈₄-FR strain showed very low levels of cross resistance with their RF values of LD₅₀ ranging from 2.4 to 11.4. This indicates that the two resistant strains probably have a different cross resistance pattern. Both fenvalerate-resistant strains showed a slight tolerance to dichlorvos and acephate, but no cross resistance to cartap.

Chou & Cheng,¹²⁾ and Chen & Sun⁷⁾ have found that the fenvalerate-selected strains of diamondback moths show high cross resistance to other pyrethroids but show low cross resistance to organophosphorus insecticides. Their cross resistance mechanism to other pyrethroids is probably the same as the resistance mechanism to pyrethroids.

It is well acknowledged that organophosphate-selected diamondback moths usually develop cross resistance to other organophosphorus compounds.^{1,5,6,12)} Noppun *et al.*¹³⁾ have reported that two phenthoate-selected strains exhibited cross resistance to dichlorvos, prothiophos and cyanophos. However, no cross resistance to fenvalerate was observed in the phenthoate-selected strains. On the other hand, Liu *et al.*⁵⁾ have reported that a diazinon-selected diamondback moth strain resistant to diazinon 15.1-fold also showed >20-fold cross resistance to pyrethroids. Further, carbosulfan- and carbosulfan-selected strains exhibited more than 30-fold cross resistance to the same pyrethroids.¹⁴⁾ The results on cross resistance of the phenthoate-selected strains¹³⁾ and of the fenvalerate-selected strains obtained in the experiment suggest a possibility of

Table 2 Slope of dosage probit mortality regression equation, LD₅₀, LD₉₅ and values of resistance factor of seven insecticides in four strains of diamondback moths at 24 hr after topical application.

Insecticide	Strain	SRE ^{a)}	LD ₅₀ (μg/g)		RF of LD ₅₀ ^{b)}	LD ₉₅ (μg/g)		RF of LD ₉₅ ^{c)}
Dichlorvos	KAR ₈₄ -FR	3.03	2770	1900 ^{e)}	1.0	(51,000) ^{d)}	8950	1.7
	KAR ₈₄	1.62	2880		1.0	(30,100)		1.0
	OKR ₈₄ -FR	1.40	4840	1170	2.4	(72,300)	16,300	5.9
	OKR ₈₄	2.08	2000		1.0	(12,300)		1.0
Phenthoate	KAR ₈₄ -FR	1.03	980	264	13.7	(39,100)	5220	49.7
	KAR ₈₄	1.58	71.5		1.0	787		1.0
	OKR ₈₄ -FR	1.85	147	204	2.4	1140	4450	3.0
	OKR ₈₄	2.09	62.5		1.0	383		1.0
Prothiophos	KAR ₈₄ -FR	1.17	3340	164	24.2	(85,300)	1430	139
	KAR ₈₄	2.53	138		1.0	614		1.0
	OKR ₈₄ -FR	1.67	1030	145	4.2	(9910)	1410	3.9
	OKR ₈₄	1.62	247		1.0	2540		1.0
Cyanophos	KAR ₈₄ -FR	1.46	18,000	435	53.4	(243,000)	2690	91.4
	KAR ₈₄	1.83	337		1.0	2660		1.0
	OKR ₈₄ -FR	1.62	2190	270	8.6	(22,600)	5200	14.2
	OKR ₈₄	2.07	256		1.0	1590		1.0
Acephate	KAR ₈₄ -FR	1.43	9820	3220	2.1	(139,000)	18,200	5.3
	KAR ₈₄	2.16	4580		1.0	(26,400)		1.0
	OKR ₈₄ -FR	2.17	7260	2540	1.0	(41,500)	16,800	0.9
	OKR ₈₄	2.13	7590		1.0	(45,000)		1.0
Methomyl	KAR ₈₄ -FR	0.84	6470	1070	37.2	(577,000)	8360	341
	KAR ₈₄	1.67	174		1.0	1690		1.0
	OKR ₈₄ -FR	1.29	2910	461	11.4	(54,800)	5190	28.7
	OKR ₈₄	1.88	256		1.0	1910		1.0
Cartap	KAR ₈₄ -FR	1.90	4020	2510	1.0	(29,400)	23,900	1.3
	KAR ₈₄	2.24	4080		1.0	(22,200)		1.0
	OKR ₈₄ -FR	1.93	2510	2100	1.1	(17,700)	20,500	1.0
	OKR ₈₄	1.86	2360		1.0	(18,000)		1.0

a-d) As in Table 1.

e) Values of LD₅₀ and LD₉₅ of KAR₈₄ and OKR₈₄ strains before selection with fenvalerate.

alternative use of some organophosphates and cartap (with no cross resistance) for control of fenvalerate-resistant diamondback moths.

3. Synergistic Effect of Various Synergists with Fenvalerate A against Fenvalerate-resistant Strains

The fenvalerate-resistant strains (KAR₈₄-FR and OKR₈₄-FR) showed 303- and 217-fold resistance to fenvalerate A compared with the susceptible counterparts (KAR₈₄ and OKR₈₄), respectively (Table 3). Treatment combined with TPP and fenvalerate A increased the

toxicity of fenvalerate A to both resistant and susceptible strains. The synergist ratio (SR) values of LD₅₀ were as high as 17.6 and 17.9 for the KAR₈₄-FR and OKR₈₄-FR strains, respectively. In the susceptible strains (KAR₈₄ and OKR₈₄), the SR values of LD₅₀ were also high (47.3 and 49.4, respectively). At LD₉₅ levels the resistant strains exhibited SR values tenfold larger than the susceptible strains. Liu *et al.*⁶⁾ have suggested that esterase inhibitors TPP and TBPT (S,S,S-tributyl phosphorotrithioate) could synergize consistently and effectively with permethrin in the resistant

Table 3 Effect of various synergists on the toxicity of fenvalerate A at 24 hr after topical application to diamondback moths.

Chemical	Strain	SRE ^{a)}	LD ₅₀ (μ g/g)	RF of LD ₅₀ ^{b)}	SR of LD ₅₀ ^{d)}	LD ₉₅ (μ g/g)	RF of LD ₅₀ ^{c)}	SR of LD ₉₅ ^{e)}
Fen. A + TPP	KAR ₈₄ -FR	0.80	9.75		17.6	(1080) ^{f)}		148
	KAR ₈₄	1.26	0.013		47.3	0.24		15.3
	OKR ₈₄ -FR	1.00	6.61		17.9	309		275
	OKR ₈₄	1.27	0.012		49.4	0.22		14.8
Fen. A + PB	KAR ₈₄ -FR	0.89	7.31		23.5	506		316
	KAR ₈₄	1.23	0.072		8.5	1.46		2.5
	OKR ₈₄ -FR	1.23	4.20		28.1	90.4		942
	OKR ₈₄	1.32	0.053		12.1	0.80		4.1
Fen. A + Sesamin	KAR ₈₄ -FR	0.79	21.78		7.9	(2670)		59.9
	KAR ₈₄	1.70	0.16		3.5	1.51		2.4
	OKR ₈₄ -FR	0.85	9.20		12.8	794		107
	OKR ₈₄	1.61	0.23		2.4	2.38		1.4
Fen. A + Sesamol	KAR ₈₄ -FR	0.86	30.13		5.7	(2520)		63.5
	KAR ₈₄	1.63	0.11		5.1	1.14		3.2
	OKR ₈₄ -FR	0.83	11.83		10.0	1090		78.1
	OKR ₈₄	1.50	0.12		4.4	1.55		2.1
Fen. A + Episesamin	KAR ₈₄ -FR	0.64	68.55		2.5	(24,700)		6.5
	KAR ₈₄	2.48	0.15		3.9	0.68		5.4
	OKR ₈₄ -FR	0.94	10.64		11.1	598		142
	OKR ₈₄	2.70	0.16		3.4	0.66		5.0
Fen. A + S-421	KAR ₈₄ -FR	0.72	89.74		1.9	(17,100)		9.4
	KAR ₈₄	1.57	0.33		1.7	3.74		1.0
	OKR ₈₄ -FR	0.98	16.44		7.2	773		110
	OKR ₈₄	2.08	0.39		1.4	2.39		1.4
Fen. A	KAR ₈₄ -FR	0.51	172	303		(160,000)	44,200	
	KAR ₈₄	2.04	0.57	1.0		3.62	1.0	
	OKR ₈₄ -FR	0.58	118	217		(85,100)	25,800	
	OKR ₈₄	2.10	0.54	1.0		3.30	1.0	

a-c) As in Table 1.

d) Synergist ratio of LD₅₀=LD₅₀ of fenvalerate A/LD₅₀ of synergized fenvalerate A.e) Synergist ratio of LD₉₅=LD₉₅ of fenvalerate A/LD₉₅ of synergized fenvalerate A.

f) Values in parenthesis are extrapolated.

strain. In contrast, Chen & Sun⁷⁾ have showed the very low synergistic effect of TPP with fenvalerate and permethrin in the mixed-field strain.

Treatment combined with PB regressed the resistance levels of the strains resistant to fenvalerate A. The SR values of LD₅₀ were 23.5 and 28.1 for the KAR₈₄-FR and OKR₈₄-FR strains, respectively. Higher synergism was obtained at LD₉₅ levels than at LD₅₀ levels. The toxicity of fenvalerate A to the susceptible strains (KAR₈₄ and OKR₈₄) also increased with

the SR values of LD₅₀ of 8.5 and 12.1, respectively. These results are similar to the ones on the synergistic experiments of some pyrethroid insecticides with PB in pyrethroid-resistant strains of diamondback moths.^{5,7,14,15)} However, Teh *et al.*¹⁶⁾ have reported no synergistic effect of PB with permethrin against Cameron Highlands' diamondback moths. On the other hand, the results of selection with a mixture of fenvalerate (racemic) and PB have indicated that diamondback moths developed resistance to this mixture within 10 genera-

tions.^{7,14,15)} Thus the possibility of using PB as a synergist for pyrethroids is fairly limited.

The other oxidase inhibitors, sesamin, sesamol, episesamin and S-421 showed less synergistic effect than PB and TPP on the KAR₈₄-FR strain. Only slight synergisms were observed at LD₅₀ levels, while at LD₉₅ levels, high synergisms of sesamin and sesamol with fenvalerate A were recorded (Table 3). However, these synergists enhanced the toxicity of fenvalerate A to the OKR₈₄-FR strain (SR values of LD₅₀ and LD₉₅ were >10 and >70, respectively). Among the synergists tested, S-421 was the least effective, and they exhibited higher synergism at LD₉₅ levels than at LD₅₀ levels.

Fenvalerate is extensively metabolized by both oxidative and esterase enzymes in plants, insects and mammals.¹⁷⁻¹⁹⁾ Pyrethroid-resistance mechanisms in the house fly, *Musca domestica*, are to reduce cuticular permeability and to enhance oxidative and hydrolytic metabolisms and insensitivity of the nervous system (*kdr*).²⁰⁾ More than 20-fold synergism of PB with fenvalerate A and the resulting decrease in LD₅₀ from more than 100 µg/g to less than 10 µg/g in the resistant strains suggest that an increase in oxidative metabolism might play a role in fenvalerate resistance in diamondback moths. Moreover, the synergism results imply that fenvalerate-resistant diamondback moths have probably accumulated more than one form of oxidases such as PB-, sesamin-, sesamol-, episesamin- and S-421-suppressable oxidases. Similarly, oxidases in the KAR₈₄-FR and OKR₈₄-FR strains are somehow different, as indicated by the different levels in synergisms with episesamin and S-421. On the other hand, metabolisms enhanced by oxidases cannot be totally accounted for >500-fold resistance in these selected strains, thus other mechanisms such as reduction in cuticular penetration and an insensitivity of the nervous system to fenvalerate might also be important for fenvalerate resistance. Hama *et al.*²¹⁾ have reported that reduced sensitivity of the central nervous system is one of the resistance mechanisms for pyrethroid resistance in diamondback moths. Further studies on cuticular penetration and insensitivity of the nervous system

are needed to reach the conclusion.

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要 約

フェンバレレート抵抗性コナガにおける交差抵抗性と共力作用

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フェンバレレートで淘汰したコナガ 2 系統 (KAR₈₄-FR, OKR₈₄-FR) はフェンバレレート (ラセミ体) に 500 倍以上の抵抗性を示し, フェンバレレート A 体 (S-異性体) や B 体 (R-異性体) にも同様な抵抗性を示した. KAR₈₄-FR 系統はフェントエート, プロチオフォス, サイアノフォス, それにメソミルに弱い交差抵抗性を示した. OKR₈₄-FR 系統はこれら殺虫剤には非常に弱い交差抵抗性を示した. フェンバレレート抵抗性の両系統はアセフェートとジクロルボスには少し耐性を示したが, カルタップには交差抵抗性を示さなかった. 両抵抗性系統に対しピペロニルブトキシド (PB) とトリフェニルフォスフェート (TPP) はフェンバレレート A 体と高い共力作用を示し, 共力作用系数は 17 以上であった. TPP とフェンバレレート A 体との共力作用は LD₅₀ 値では感受性系統 (KAR₈₄, OKR₈₄) で抵抗性系統より高かったが, LD₉₅ 値では抵抗性系統のほうが感受性系統より高かった. 一方, セサミン, セサモール, エピセサミンや S-421 とフェンバレレート A 体との共力作用は KAR₈₄-FR 系統では低かったが, OKR₈₄-FR 系統では高かった.