Original Article

Metabolism of Fenitrothion in the Resistant and Susceptible Diamondback Moth, *Plutella xylostella* L. (Lepidoptera : Yponomeutidae)*

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In vivo metabolism of [methoxy-¹⁴C]fenitrothion topically applied to the organophosphorus-resistant (Mizobe) and -susceptible (Sapporo) strains of the diamondback moth was examined. There was no difference in the rate of penetration between the two strains. Significant differences were found in the formation of internal metabolites, *i.e.*, fenitroxon levels were 3 to 4 times higher in the Sapporo strain, while the levels of dimethyl phosphate, a hydrolysis product of fenitroxon, were 2 times higher in the Mizobe strain. To confirm the differences *in vitro* metabolism of [methoxy-¹⁴C]fenitroxon was examined. In both strains, fenitroxon was detoxicated mainly by the hydrolysis in the soluble fraction, but the hydrolytic activity was 2 times higher in the Mizobe strain. Based on these results, it was proved that the increased fenitroxon hydrolysis in the soluble fraction plays an important role for fenitrothion resistance in the Mizobe strain.

INTRODUCTION

The diamondback moth, *Plutella xylostella* L., is a serious pest of cruciferous vegetables, and the insecticide resistance problem has occurred in many parts of the world.¹⁻⁵⁾ With respect to the resistance to organophosphorus (OP) insecticides, several mechanisms have been identified, including enhanced detoxication by non-specific esterases⁶⁾ and glutathione *S*-transferases,^{7, 8)} and insensitivity of acetylcholinesterase.⁹⁻¹¹⁾ In contrast with these detailed enzymatic studies, information on the OP insecticides metabolism is very limited. The sole study was done by Noppun *et al.*¹²⁾ Their *in vivo* study using ¹⁴C-phenthoate revealed that metabolism is not a major factor, but the reduced cuticular penetration is important for the resistance in the phenthoate-resistant OKR-R and OSS-R strains of the diamondback moth.

In order to elucidate the resistant mechanism in the diamondback moth, the present paper examined *in vivo* and *in vitro* metabolism of ¹⁴C-fenitrothion and ¹⁴C-fenitroxon (an actual anticholinesterase metabolite of fenitrothion) in the larvae of OP-resistant and susceptible

strains of the diamondback moth.

MATERIALS AND METHODS

1. Insects

An OP-resistant (Mizobe) and susceptible (Sapporo) strains of the diamondback moth were used. Origins of the two strains and rearing conditions were described previously.¹¹⁾ Fourth instar larvae were used.

2. Chemicals

Fenitrothion (*O, O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothioate), fenitroxon (*O, O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphate), dimethyl phosphorothioic acid, dimethyl phosphoric acid, desmethyl fenitrothion (*O*-methyl *O*-hydrogen *O*-3-methyl-4-nitrophenyl phosphorothioate), *S*-methyl glutathione, [methoxy-¹⁴C]fenitrothion (12,364 dpm/ μ g) and [methoxy-¹⁴C]-fenitroxon (13,336 dpm/ μ g) were obtained from Sumitomo Chemical Co., Ltd. (Osaka, Japan). Purities of these chemicals were more than 98% by thin-layer chromatography (TLC) analysis. All other chemicals were commercially available.

3. Toxicity Test

Susceptibility to fenitrothion and fenitroxon was determined by the topical application. An acetone solution (0.25 μ l) of OP insecticides was applied to the dorsal

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region of individual fourth instar larvae anaesthetized with CO₂ using a micro-applicator (Burkard, England). Five concentrations of each OP insecticides were evaluated using 10 larvae per concentration and replicated three times. Treated larvae were transferred to a plastic Petri dish (9 cm dia. × 2 cm ht.) containing the cut leaf of rapeseed and kept at 24±2°C. Mortality was counted 24 hr after the treatment. LD₅₀ values were determined by probit analysis.

4. *In Vivo Metabolism of [Methoxy-¹⁴C]fenitrothion*

In vivo metabolism of [methoxy-¹⁴C]fenitrothion was carried out based on the methods of Konno & Shishido.¹³⁾ Individual larvae were topically treated with 1 µg of [methoxy-¹⁴C]fenitrothion in 0.25 µl of acetone. Groups of 10 treated larvae were kept at 25°C in 10 ml glass tubes. After an appropriate time, the surface of the larvae and the glass tube were rinsed with 4 ml of acetone (the external fraction). The washed larvae were homogenized and extracted with 5 ml of aqueous 80% acetone solution (the internal fraction). After the acetone extracts were evaporated to near dryness *in vacuo*, radioactive metabolites in the internal and external fractions were partitioned by extraction with chloroform and water, and radioactivity was determined by liquid scintillation counting (LSC). Metabolites in the water-soluble and chloroform-soluble fractions were further analyzed by TLC (60-F₂₅₄, Merck) and autoradiography with authentic standards. Solvent systems, *R_f* values of metabolites and the detection methods of metabolites on TLC were described previously.¹³⁾ Radioactive compounds scrapped off from TLC plates were quantified by LSC.

5. *In Vitro Metabolism of [Methoxy-¹⁴C]fenitroxon*

In vitro metabolism of [methoxy-¹⁴C]fenitroxon was carried out based on the methods of Konno & Shishido.¹³⁾ Whole bodies of larvae were homogenized (5% w/v) with 1 mM EDTA-5 mM 2-mercaptoethanol-0.1 M phosphate buffer, pH 7.4 in an ice bath. The homogenates were centrifuged at 600×*g* for 10 min to remove nuclei and debris, followed by 10,000×*g* for 10 min to remove mitochondria. Supernatant fractions were further centrifuged at 105,000×*g* for 60 min to separate microsomes and soluble fraction. Protein concentration of the mitochondria, microsomes and soluble fraction in the Sapporo strain was 0.33, 0.44 and 4.1 mg/ml, and that in the Mizobe strain was 0.31, 0.42 and 3.9 mg/ml, respectively. A reaction mixture consisting of 1 ml of each fraction and 10 µg of [methoxy-¹⁴C]fenitroxon (in 10 µl EtOH) was incubated at 27°C. Because the purpose of the *in vitro* study was focused only on enzymatic hydrolysis and sequestration, no other reagents such as NADPH and GSH were added to the above reaction mixture. After an appropriate time, 1 ml of

14% trichloroacetic acid was added to precipitate protein, and then 3 ml of chloroform was added to remove unchanged fenitroxon. The chloroform layer, aqueous layer and precipitate protein were separated by centrifugation (3000×*g*, 10 min). Radiocarbon of the aqueous layer possibly from enzymatic hydrolysis was measured by LSC. The protein precipitate was solubilized with 1 ml of NCS (Amersham), and radiocarbon bound to protein (sequestration) was measured by LSC. All data are the average of three replicates. Protein concentration was determined according to the method of Bradford.¹⁴⁾

RESULTS AND DISCUSSION

Susceptibility of the Mizobe and Sapporo strains of the diamondback moth to fenitrothion and fenitroxon are shown in Table 1. The Mizobe strain was highly resistant to fenitrothion (>42-fold) and fenitroxon (81-fold). *In vivo* metabolism of fenitrothion was carried out using 1 µg/larva of ¹⁴C-fenitrothion. At this dose, the larvae showed no symptoms of intoxication 2 hr after the treatment. The fate of fenitrothion topically applied to the diamondback moth is shown in Fig. 1. There was no significant differences in the recovery of radiocarbon from the internal and external fractions between the two strains. Particularly, penetration rates which were estimated from the amount of radioactivity recovered from the external chloroform-soluble fraction were almost same between the two strains.

However, a significant difference between the two strains was found in the formation of internal metabolites of ¹⁴C-fenitrothion. As shown in Table 2, internal levels of fenitroxon were 3 to 4 times higher in the Sapporo strain than in the Mizobe strain. Internal levels of dimethyl phosphate, which is a hydrolysis product of fenitroxon, were 2 times higher in the Mizobe strain, indicating a faster detoxication of fenitroxon in the Mizobe strain than in the Sapporo strain. Internal levels of dimethyl phosphorothioic acid were almost same in the two strains. Desmethyl fenitrothion and *S*-methyl glutathione were very little and there was no differences between the two strains.

Table 1 Toxicity of fenitrothion and fenitroxon to resistant (Mizobe) and susceptible (Sapporo) strains of the diamondback moth.

Insecticides	LD ₅₀ (µg/larva) ^{a)}		RR ^{b)}
	Sapporo	Mizobe	
Fenitrothion	2.4 (1.9-3.1)	>100	>42
Fenitroxon	0.47 (0.29-0.73)	38 (30-48)	81

^{a)} Figures in parentheses indicate 95% confidence limits.

^{b)} RR (resistance ratio)=LD₅₀ of the Mizobe strain/LD₅₀ of the Sapporo strain.

Fig. 1 Fate of [methoxy- ^{14}C]fenitrothion topically applied to the susceptible (Sapporo) and resistant (Mizobe) strains of the diamondback moth.

"External" and "Internal" are explained in the text. $\triangle$ (Sapporo) and $\blacktriangle$ (Mizobe): chloroform-soluble fraction, $\square$ (Sapporo) and $\blacksquare$ (Mizobe): water-soluble fraction.

Table 2 Internal metabolites of [methoxy- ^{14}C]fenitrothion topically applied to the resistant (Mizobe) and susceptible (Sapporo) strains of the diamondback moth.^{a)}

Metabolite	Percentage of radioactivity			
	Hours after application			
	1		2	
	Sapporo	Mizobe	Sapporo	Mizobe
Fenitrothion	4.9 $\pm$ 3.2 ^{b,c)}	4.7 $\pm$ 2.2	4.6 $\pm$ 1.7	4.7 $\pm$ 2.5
Fenitroxon	0.7 $\pm$ 0.4	0.2 $\pm$ 0.1*	0.9 $\pm$ 0.4	0.3 $\pm$ 0.2*
Dimethyl phosphorothioic acid	6.4 $\pm$ 3.4	5.0 $\pm$ 4.2	7.9 $\pm$ 4.6	6.2 $\pm$ 1.7
Dimethyl phosphoric acid	1.8 $\pm$ 0.8	3.4 $\pm$ 0.5*	2.5 $\pm$ 1.6	5.7 $\pm$ 2.0*
Desmethyl fenitrothion	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1
S-Methyl glutathione	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.5 $\pm$ 0.2	0.3 $\pm$ 0.2

^{a)} Applied doses: 1 μg /larva.

^{b)} Mean $\pm$ S.E. of three experiments.

^{c)} Mean activity of the Mizobe strain followed by * is significantly different from that of the Sapporo strain ($p < 0.05$; Student's t -test).

Time plots of hydrolysis and sequestration of ^{14}C -fenitroxon supported the above results (Fig. 2). In both strains, fenitroxon was detoxicated mainly by the hydrolysis in the soluble fraction, and the hydrolytic activity was 2 times higher in the Mizobe strain than in the Sapporo strain. On the contrary, the hydrolytic activities in microsomes and mitochondria fractions and the sequestering activity in all fractions were low and very low, respectively, and there was no significant differences between the two strains.

In our previous study,¹¹⁾ we revealed that the sensitivity of acetylcholinesterase (AChE) to fenitroxon in the Mizobe strain was 5.6-fold lower than that in the Sapporo strain. It was concluded that the fenitrothion resistance in the Mizobe strain was attributed to dual factors, i.e., the increased fenitroxon hydrolysis and the

AChE insensitivity.

We chose ^{14}C -fenitrothion in this metabolism study, although fenitrothion is not registered for cruciferous vegetables in Japan. There are two reasons for this choice, i.e., (i) the purpose of this study is to know the basic metabolic ability to OP insecticides in the diamondback moth; and (ii) to compare the fenitroxon detoxication activity of the diamondback moth with those of other insects. Figure 3 summarized the fenitroxon detoxication activity in various insects and mite, including the American cockroach, *Periplaneta americana* (Konno, unpublished data), the rice stem borer, *Chilo suppressalis*,¹⁵⁾ the caddisfly, *Cheumatopsyche brevilineata*,¹⁶⁾ the cotton aphid, *Aphis gossypii*,¹⁷⁾ and the bulb mite, *Ryzoglyphus robini*.¹⁸⁾ Apparently, the Mizobe strain has the highest detoxicating activity

Fig. 2 Time plots of hydrolysis and sequestration of [methoxy- ^{14}C]fenitroxon incubated with each fraction from the susceptible (Sapporo) and resistant (Mizobe) strains of the diamondback moth. Fenitroxon hydrolysis by the soluble fraction (●), microsomes (■), and mitochondria (▲); fenitroxon sequestration by the soluble fraction (○), microsomes (□), and mitochondria (△).

Fig. 3 Comparison of fenitroxon detoxication activities in various insects and mite. All data were of the soluble fractions and were measured by the methods of Konno & Shishido.¹⁵⁾ Except for American cockroach (fat body), whole bodies were used for enzyme preparation. OP-R and OP-S in parentheses means OP-resistant and OP-susceptible strains, respectively.

among the insects and mite tested, and even the Sapporo strain has a higher activity than those of the OP-resistant rice stem borer, caddisfly, cotton aphid and bulb mite, suggesting that the diamondback moth is naturally an OP insecticide-tolerant insect. Considering this fact, the easy and rapid development of the high resistance to OP insecticides in the diamondback moth is best understandable.

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要 約

抵抗性および感受性コナガ幼虫におけるフェニトロキソンの代謝

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コナガ幼虫における¹⁴C-フェニトロキソンの *in vivo* 代謝を抵抗性系統と感受性系統間で比較した。薬剤の皮膚透過性、体内代謝物の総量、および体外排泄物の総量には有為な差がみられなかったが、体内代謝物の組成割合に差が認められた。すなわち抵抗性系統では感受性系統に比べてフェニトロキソンの生体内活性毒物体であるフェニトロオクソンが有意に少なく、またフェニトロオクソンの分解物であるジメチルリン酸については逆に抵抗性系統のほうが感受性系統より有意に多かった。¹⁴C-フェニトロオクソンの *in vitro* 代謝においても、抵抗性系統では感受性系統に比べてフェニトロオクソンを速やかに解毒した。以上の結果、フェニトロオクソン解毒活性の増大が、コナガのフェニトロキソン抵抗性の要因の一つであることが明らかになった。