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Original Article

Cuticular Penetration and Metabolism of Phenthoate in the Resistant and Susceptible Diamondback Moth, *Plutella xylostella* L.*

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The study on the cuticular penetration of [methoxy-^{14}C]phenthoate in the diamondback moth showed lower rates of cuticular penetration, lower amounts of radioactivity in the insect body and higher excretion rates of phenthoate in the phenthoate-resistant strains (OSS-R and OKR-R) than in the susceptible strains (OSS and OKR). An *in vivo* metabolism study using [methoxy-^{14}C]phenthoate revealed no apparent difference between resistant and susceptible strains in overall rates of metabolism of [methoxy-^{14}C]phenthoate. The $^{14}CO_2$ produced by treated insects was too small in amounts to be considered as a major product of phenthoate metabolism. Such results of the study indicated that reduced cuticular penetration of phenthoate is one of the important resistance mechanisms in the diamondback moth, *Plutella xylostella* L.

INTRODUCTION

The authors have reported on the development of resistance to phenthoate in the diamondback moth, *Plutella xylostella* L., as a result of selection with phenthoate.[1]

The insect cuticle is generally adapted to act as a primary barrier for penetration of insecticides into the insect body.[2] Reduced penetration of insecticides through the cuticle has been claimed to be an important factor for insecticide resistance in a number of insect species. In diazinon-resistant strains of houseflies, resistance was associated with delayed penetration of diazinon.[3,4] Resistance due to reduced rate of cuticular penetration of insecticides has also been reported in *Aedes aegypti* (L.),[5] *Euxesta notata* WIESDEMANN,[6] *Heliothis virescens* (F.),[7] *Culex tarsalis* L.,[8] and *Panonychus citri* MCGREGOR.[9]

In order to clarify underlying mechanisms for phenthoate resistance in the diamondback moth, the present study examined rates of cuticular penetration and *in vivo* metabolism of [methoxy-^{14}C]phenthoate in the larvae of resistant and susceptible strains of the diamondback moth.

MATERIALS AND METHODS

1. *Insect Strains*

Two phenthoate-selected strains (OSS-R and OKR-R) of the diamondback moth developed by laboratory selection of OSS and OKR strains, respectively[1] were used in this experiment. The strains were further selected by phenthoate for higher resistance to phenthoate over four generations. Non-selected insects of both strains (OSS and OKR) were also used for comparison. Insects were reared in constant environment (25°C, 16L: 8D) as previously reported.[10,11] LD_{10} and LD_{50} values of these strains to phenthoate are listed in Table 1.

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2. Synthesis of the [Methoxy-^{14}C]phenthoate

[Methoxy-^{14}C]phenthoate was synthesized according to the methods described by Fletcher *et al.*,[12] Hirose *et al.*[13] and Gasser.[14] One mmol of anhydrous methanol containing 1 mCi of ^{14}C-labelled methanol and phosphorous pentasulfide (16.3 mg) were refluxed in anhydrous toluene (0.5 ml) at room temperature for 2 hr. The resultant [methoxy-^{14}C]-*O,O*-dimethyl dithiophosphoric acid was treated with ammonia gas, resulting in ammonium [methoxy-^{14}C]-*O,O*-dimethyl phosphordithioate. The precipitate was dissolved in acetone (0.5 ml). Ethyl α-bromophenylacetate (35.6 mg) was added to the acetone solution and stirred at room temperature for 3 hr and at 45°C for an additional hour. Acetone was removed by a rotary evaporator at 40°C, and the resultant was dissolved in 1 ml of benzene. The benzene solution was washed with 1 ml of saturated sodium bicarbonate solution, and then again with an equal volume of distilled water twice. The benzene solution was dehydrated with sodium sulfate overnight. The solution was concentrated and purified by thin-layer chromatography (Merck F_{254} TLC plate, solvent system, *n*-hexane : acetone, 4 : 1, v/v). A portion of [methoxy-^{14}C]phenthoate on the plate (*Rf* 0.4–0.5) was scrapped and extracted with acetone to produce [methoxy-^{14}C]phenthoate (5.2 mg, 83% yield with a specific activity of 14,248 dpm/μg and a radiochemical purity of 98% on TLC).

3. Determination of $^{14}CO_2$ Expiration

Acetone solutions of the labelled phenthoate were prepared to give equitoxic doses of LD_{10} (OSS: 0.028, OKR: 0.038, OSS-R: 0.527 and OKR-R: 1.02 μg/insect) and LD_{50} (OSS: 0.102, OKR: 0.160, OSS-R: 15.60 and OKR-R: 32.64 μg/insect). A 0.52 μl droplet of phenthoate solution was topically applied on the dorsal segments of thorax of larvae by a micro-applicator (Kiya Seisakusho Ltd., Tokyo).[10] After evaporation of acetone, 5–20 larvae were placed in a 20 ml scintillation vial and subsequently maintained in a water bath at 25°C. The vials were connected to the apparatus to determine the amount of $^{14}CO_2$ which were set according to the method described by Brempong-Yeboah *et al.*[15]

4. Determination of Cuticular Penetration of [Methoxy-^{14}C]phenthoate

A droplet of acetone solution (0.52 μl) containing equitoxic doses of LD_{10} or LD_{50} or equidoses of 0.527 μg/insect for OSS and OSS-R strains, or 1.02 μg/insect for OKR and OKR-R strains, was applied in the same manner as above. The treated larvae (5–20 larvae) were placed in the scintillation vial and maintained in a water bath (25°C). Fifteen (15), 30 min, 1, 2, 4, 8, 16, and 24 hr after treatment the larvae were anaesthetized with CO_2, transferred to a funnel with a metal screen and rinsed with 6 ml Aquasol-2® (New England Nuclear Co. Ltd., England). After removal of the larvae, the funnel was rinsed with an additional 1 ml Aquasol-2®. Total radioactivity in the combined 7 ml Aquasol-2® was used for determination of the unpenetrated quantity. The vial with insects in was filled with 5 ml Aquasol-2® and the radioactivity excreted was counted. The rinsed insects were combusted thereafter by an Aloka Sample Oxidizer (Aloka ASC 113, Aloka Co. Ltd., Tokyo). $^{14}CO_2$ gas released by combustion was trapped in a mixture of Oxysorb TM-CO_2® and Oxyprep-2® (5 : 7, v/v) (New England Nuclear Co. Ltd., England). The amount of radioactivity was taken as internal quantities in the insects. The experiment was replicated three times.

5. In Vivo Metabolism of [Methoxy-^{14}C]phenthoate

Twenty to a hundred larvae were treated with 0.52 μl of LD_{10}, LD_{50} or equidoses, and maintained as above. Twenty four hours after treatment, the insects were anaesthetized, washed with benzene (5 ml), and again washed the holding funnel with benzene (1 ml). One hundred μl of combined benzene (unpenetrated quantity) was counted for the ratioactivity by the liquid scintillation system (Aloka LSC-700). The remainder was evaporated to dryness, taken up in a minimum amount of methanol, and spotted on TLC (0.25 mm silica gel, F_{254}, Merck, Darmstadt, W. Germany). The washed insects were homogenized and extracted with 3×2 ml of methanol using a motor-driven Potter-Elvehjen glass homogenizer. After centrifuging at 2500 rpm for 10 min, the supernatant was collected. One

hundred μl supernatant (internal quantity) was taken and the radioactivity was counted. The remaining supernatant was evaporated to dryness, taken up in methanol, and spotted on a TLC plate in the same manner as done with the unpenetrated quantity. Two milliliters methanol was poured in three portions to the vial with insects in to extract the radioactivity that was excreted and/or rubbed off the insects. The extracted methanol (excreted quantity) was combined, homogenized by a glass homogenizer, centrifuged and 100 μl methanol extraction was counted for radioactivity. The remainder was evaporated and spotted on TLC as done with the unpenetrated quantity. The unextractable radiolabelled residue in the insects and excreta was burnt by a sample oxidizer and counted by LSC to determine the amount of unextractable radioactivity.

The TLC plate was developed with acetonitrile: water: ammonia (8: 1.8: 0.2, v/v) (Takade *et al.*).[16] Localization of metabolites on the TLC plates was detected by autoradiography (by exposing TLC plates to X-ray film (Fuji Photo Film Co. Ltd., Tokyo) for one month). Authentic compounds were co-chromatographed with samples and their *Rf* was detected under a 254 nm wavelength light. Compounds **IV** and **VII** (Table 5) were detected following the method reported by Harnes and Isherwood.[17] Each radioactive spot was scrapped into a scintillation vial and the amount of radioactivity in each spot was measured on the LSC after addition 5 ml ACS-II® scintillator cocktail (Amersham Corporation, U.S.A.). The radioactive spots were identified by comparing their *Rf* values with those of authentic compounds (Table 5). The experiment was replicated three times.

RESULTS AND DISCUSSION

1. *Amount of $^{14}CO_2$ Expiration*

Table 1 shows $^{14}CO_2$ produced by treated insects during 24 hr after topical application of [methoxy-^{14}C]phenthoate. The amount of $^{14}CO_2$ was not different between treated insects and the control at both LD_{10} and LD_{50} doses. The result suggested that the $^{14}CO_2$ was too small in amounts to be considered as a significant product of phenthoate metabolism by the diamondback moth.

2. *Cuticular Penetration of [Methoxy-^{14}C]phenthoate*

Penetration and excretion curves of [methoxy-^{14}C]phenthoate are shown in Fig. 1. Differences were consistent between the resistant (OSS-R and OKR-R) and susceptible (OSS and OKR) strains at each dose level.

The values t_{10} and t_{50} calculated according to the method of Olson and O'Brien[18] are shown in Table 2. Times required for 10% (t_{10}) and 50% (t_{50}) of applied doses to penetrate the cuticle of the insects were shorter in susceptible strains than in resistant strains. By comparison, t_{10} values at LD_{10}, LD_{50} and an equidose of 0.527 μg/insect were 1.2, 4.7 and 1.1 times higher in the OSS-R strain than in the OSS strain, respectively. The same pattern was also observed in the OKR strain, whose t_{10} values at LD_{10}, LD_{50} and an equidose of 1.02 μg/insect were 1.9, 2.4 and 2.0 times higher than those of the OKR strain, respectively. Moreover, the resistant strains also showed the same penetration pattern at t_{50} values of equitoxic doses and equidoses as at the t_{10} level. Though

Table 1 Percent $^{14}CO_2$ produced by insects at 24 hr after treatment with [methoxy-^{14}C]-phenthoate at LD_{10} and LD_{50} doses.

Strain	LD_{10}[a] (μg/insect)	LD_{10}		LD_{50}[a] (μg/insect)	LD_{50}	
		Treat.	Cont.[b]		Treat.	Cont.[b]
OSS	0.028	1.6±0.5	—	0.102	2.8±1.5	3.6±1.5
OSS-R	0.527	1.2±0.5	2.3±1.6	15.60	1.3±0.6	1.3±0.7
OKR	0.038	3.0±1.2	3.1±1.0	0.160	2.6±1.1	3.0±1.6
OKR-R	1.02	0.9±0.4	1.5±1.2	32.64	1.3±0.7	1.4±0.7

[a] Data were cited from Noppun *et al.*[1]

[b] Filter paper treated with [methoxy-^{14}C]phenthoate.

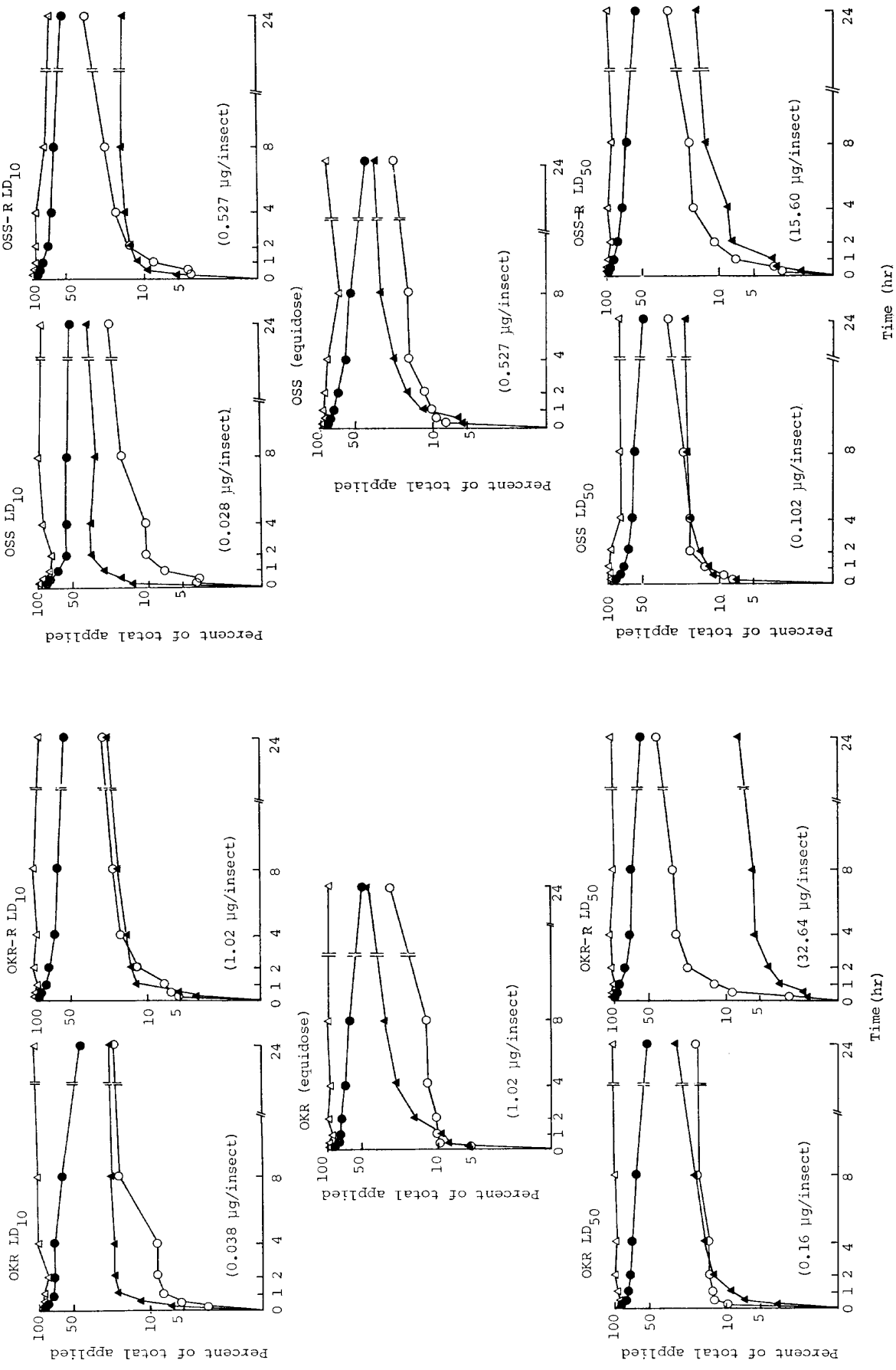


Fig. 1 Penetration and excretion of [methoxy-^{14}C]phenthoate at various times after topical application to four strains of the diamondback moth.

△: Total recovery, ●: Unpenetrated quantities, ▲: Internal quantities, ○: Excreted quantities.

Table 2 Times in min required for 10% (t_{10}) and 50% (t_{50}) of applied doses of 0.527 μg/insect, 1.02 μg/insect, LD_{10} and LD_{50} to penetrate the cuticle of the diamondback moth.[a)]

Dose		Strain		R/S	Strain		R/S
		OSS	OSS-R		OKR	OKR-R	
LD_{10}	t_{10}	12.0±0.04	14.3±0.9	1.2	15.3±0.2	29.0±0.8	1.9
	t_{50}	663.3±27.8	>1440	—[b)]	1302.0±102.8	>1440	—[b)]
LD_{50}	t_{10}	12.3±1.3	58.3±2.4	4.7	11.3±0.5	27.5±3.5	2.4
	t_{50}	1347.5±65.5	>1440	—[b)]	1368.8±100.8	>1440	—[b)]
0.527	t_{10}	12.7±1.1	14.3±0.9	1.1	—	—	
	t_{50}	657.0±155.7	>1440	—[b)]	—	—	
1.02	t_{10}	—	—		14.8±0.3	29.0±0.8	2.0
	t_{50}	—	—		967.2±88.9	>1440	—[b)]

a) t_{10} and t_{50} values were calculated as means ± standard deviations using graphs of individual replicate.
b) Data could not be calculated.

Table 3 Times in min required for 5% (Rt_5) and 10% (Rt_{10}) of applied doses of 0.527 μg/insect, 1.02 μg/insect, LD_{10} and LD_{50} to be recovered from the inside of the diamondback moth body.[a)]

Dose		Strain		R/S	Strain		R/S
		OSS	OSS-R		OKR	OKR-R	
LD_{10}	Rt_5	10.3±0.9	15.0±0.8	1.5	13.3±0.9	24.2±3.5	1.8
	Rt_{10}	12.2±0.7	45.0±11.0	3.7	29.2±0.6	49.0±4.3	1.7
LD_{50}	Rt_5	16.8±4.1	91.8±19.9	5.5	21.9±5.7	291.1±95.9	13.3
	Rt_{10}	33.3±9.4	226.3±114.4	6.8	70.8±8.6	678.8±280.6	9.6
0.527	Rt_5	14.5±0.7	15.0±0.8	1.0	—	—	
	Rt_{10}	52.5±5.3	45.0±11.0	0.9	—	—	
1.02	Rt_5	—	—		18.0±4.3	24.2±3.5	1.3
	Rt_{10}	—	—		66.3±10.8	49.0±4.3	0.7

a) Rt_5 and Rt_{10} values were calculated as means ± standard deviations using graphs of individual replicate.

t_{50} values for resistant strains were very difficult to calculate, the result indicated that the values exceeded 24 hr (>1440 min) and were apparently higher than those of susceptible counterparts (<23 hr).

Times required for 5% (Rt_5) and 10% (Rt_{10}) of the applied doses to be recovered from the inside of the insect body are presented in Table 3. Times required for 5% and 10% recovery were chosen, since >20% recovery was unattainable in most of the treatments. There were significant differences in amounts of internally recovered radioactivity between resistant and susceptible strains. The OSS-R strain showed Rt_5 and Rt_{10} values 1.5 and 3.7, and 5.5 and 6.8 times higher than the OSS strain at LD_{10} and LD_{50} levels, respectively. Similarly, the OKR-R strain's Rt_5 values were 1.8×(LD_{10}) and 13.3×(LD_{50}), and Rt_{10} 1.7×(LD_{10}) and 9.6×(LD_{50}) higher than the OKR strain's. However, such a tendency could not be observed at equidoses (0.527 and 1.02 μg/insect). This might be due to the fact, at least in part, that at equidoses all susceptible insects were completely killed with no excretion, and radioactivity was mostly kept within the insects.

Table 4 shows times required for 10% (Et_{10}) and 20% (Et_{20}) of applied doses to be excreted by treated insects. Ten and 20%

Table 4 Times in min required for 10% (Et_{10}) and 20% (Et_{20}) of applied doses of 0.527 μg/insect, 1.02 μg/insect, LD_{10} and LD_{20} to be excreted by the diamondback moth.[a)]

Dose		Strain		R/S	Strain		R/S
		OSS	OSS-R		OKR	OKR-R	
LD_{10}	Et_{10}	131.8±46.2	86.3±5.4	0.7	157.3±1.4	105.6±15.2	0.7
	Et_{20}	928.0±268.9	508.8±118.2	0.6	>1440	560.0±299.1	—[b)]
LD_{50}	Et_{10}	35.0±18.7	63.8±30.9	1.8	25.0±10.2	39.2±8.3	1.6
	Et_{20}	252.2±163.4	410.0±125.7	1.6	>1440	111.0±6.4	—[b)]
0.527	Et_{10}	111.3±33.9	86.3±5.4	0.8	—	—	
	Et_{20}	716.7±520.3	508.8±118.4	0.7	—	—	
1.02	Et_{10}	—	—		65.5±39.5	105.6±15.2	1.6
	Et_{20}	—	—		>1440	560.0±298.8	—[b)]

a) Et_{10} and Et_{20} values were calculated as means ± standard deviations using graphs of individual replicate.

b) Data could not be calculated.

levels were chosen as a matter of convenience because in most of treatments 50% excretion were unattainable. As for LD_{10}, the resistant strains showed faster rate of excretion than the susceptible strains at both the Et_{10} and Et_{20} levels. On the other hand, at LD_{50} doses, the susceptible strains exhibited faster Et_{10} and Et_{20} than the resistant strains, with the exception of Et_{20} of the OKR-R strain (111±6.4 min), which was faster than that of the OKR strain (>1440 min). At an equidose of 0.527 μg/insect, the OSS-R strain exhibited faster Et_{10} and Et_{20} than the OSS strain. Results at an equidose of 1.02 μg/insect for the OKR and OKR-R strains indicated that the OKR strain exhibited faster Et_{10} (65.5±39.5 min) than the OKR-R strain (105.6±15.2 min). The OKR-R strain, however, showed faster Et_{20} (560±298.8 min) than its OKR counterpart. Results of excretion did not indicate clear-cut differences in rates of excretion between the resistant and susceptible strains. However, Fig. 1 showed a tendency that in all cases, the resistant strains excreted larger amounts while the susceptible strains retained larger amounts within the body.

Results of this experiment indicate that there may be three different behaviours of ^{14}C-phenthoate in resistant and susceptible strains. First, penetration rates were slower in resistant strains, second, resistant strains maintained smaller amounts of radioactivity in the body, and third, the excretion rates were higher in the resistant strains.

3. *In Vivo Metabolism of [Methoxy-^{14}C]phenthoate*

Data for the recovery of [methoxy-^{14}C]-phenthoate 24 hr after topical application to susceptible and resistant strains of the diamondback moth are presented in Table 6. The total recovery of radioactivity ranged from 64.1 to 95.8%, and more than 40% was presented in the unpenetrated quantity. In the internal quantity of insects treated at equidoses and equitoxic doses, absorption rates of radioactivity were apparently different between the resistant and susceptible strains. Thus, it appears that reduced penetration is a significant factor to contribute to the >100-fold stronger resistance of the resistant strains.

Results of phenthoate metabolism in the resistant and susceptible strains are presented in Tables 7–10. Metabolites of [methoxy-^{14}C]-phenthoate were identified in various amounts in internal and excreted quantities, but very small amounts in unpenetrated quantity. Since the metabolites found in the unpenetrated quantity were always less than 10%, they were included in total metabolites. Results showed that there was no apparent difference in amounts of metabolites found in the unpenetrated quantity between the resistant and susceptible strains. Metabolites of phenthoate metabolism were: phenthoate oxon (**II**), demethyl phenthoate (**III**), *O,O*-dimethyl phos-

Table 5 TLC properties of phenthoate and metabolites on silica gel F_{254}, Merck.[a)]

No.	Structure	*Rf*
I	$(CH_3O)_2P(S)SCHCOOC_2H_5$ C_6H_5	0.95
II	$(CH_3O)_2P(O)SCHCOOC_2H_5$ C_6H_5	0.92
III	$(HO)(CH_3O)P(S)SCHCOOC_2H_5$ C_6H_5	0.72
IV	$(CH_3O)_2P(S)SH$	0.62
V	$(CH_3O)_2P(S)SCHCOOH$ C_6H_5	0.56
VI	$(HO)(CH_3O)P(O)SCHCOOC_2H_5$ C_6H_5	0.48
VII	$(CH_3O)_2P(S)OH$	0.41
VIII	$(HO)(CH_3O)P(S)SCHCOOH$ C_6H_5	0.31
IX	Unknown A	0.18
X	Unknown B	0.14
XI	Unknown C	0.10

a) The solvent system had the following compositions; acetonitrile : water : ammonium hydroxide (8 : 1.8 : 0.2).

phorodithioic acid (**IV**), phenthoate acid (**V**), demethyl phenthoate oxon acid (**VI**), phosphorothioic acid (**VII**), demethyl phenthoate acid (**VIII**), unknown A (**IX**), unknown B (**X**) and unknown C (**XI**) (see Table 5 for chemical structures).

Quantitatively, a significant difference between the resistant and susceptible strains was in amounts of metabolites detected in the excreted quantity that contained large amounts of radioactivity in the susceptible strains treated at equitoxic doses (Tables 7–10). On the contrary, the resistant strains had larger amounts of metabolites when treated at equidoses (Tables 7 and 8). The reason could be explained by the fact that equidoses killed susceptible insects completely, so no excretion occurred. Among metabolites found in the excreted quantity, compound **VIII** was the major metabolite.

Owing to variability in the internal quantity, distinct differences in phenthoate metabolism between the resistant and susceptible strains were found only when the OSS and OSS-R strains were treated at LD_{10} (Table 7). The OSS-R strain showed greater phenthoate metabolism than the OSS strain. However, such a tendency was not detected when the insects were treated at LD_{50} and the OKR and OKR-R strains were treated at LD_{10} (Tables 8–10). Consequently, there was also no apparent difference in phenthoate metabolism between the resistant and susceptible strains when they were treated at equidoses of 0.527 and 1.02 μg/insect (Tables 7 and 8). The results of phenthoate metabolism in the internal quantity

Table 6 Distribution of radioactivity 24 hr after topical application of [methoxy-^{14}C]-phenthoate to susceptible and resistant diamondback moths.

Strains	Dose (μg/insect)	Percentage of recovered radioactivity[a)]				Total rec. (%)
		Unpen.	Excre.	Inter.	Unext.	
OSS	0.028	40.8±12.9[b)]	33.2±8.4	18.5±4.0	7.5±2.2	75.9±18.6
	0.102	39.8±5.5	43.9±3.3	13.3±4.0	3.0±0.6	69.7±18.1
	0.527	49.6±4.1	37.7±5.1	11.2±1.8	1.5±0.3	84.4±11.7
OKR	0.038	39.7±2.6	39.2±2.2	14.6±0.3	6.5±0.7	64.1±8.3
	0.160	51.4±7.4	31.4±6.3	14.6±1.4	2.6±0.0	80.2±12.5
	1.02	51.6±2.8	29.6±5.9	16.7±3.9	2.1±0.5	94.3±6.7
OSS-R	0.527	46.5±2.9	46.7±1.7	5.6±1.4	1.2±0.1	95.8±7.0
	15.60	54.7±3.4	34.5±3.5	9.9±0.0	0.9±0.1	91.4±0.1
OKR-R	1.02	49.0±2.6	43.4±2.8	6.6±2.3	1.0±0.2	92.7±0.1
	32.64	51.5±2.8	38.3±5.8	9.6±3.9	0.6±0.0	95.3±4.2

a) Each figure was expressed as a percentage of total recovered radioactivity.

b) Means ± standard deviations.

Table 7 Metabolites recovered from OSS and OSS-R strains of the diamondback moth 24 hr after topical application of [methoxy-^{14}C]phenthoate (LD_{10} and 0.527 μg/insect).

Met.[a]	Percentage of radioactivity								
	OSS (0.028 μg/insect)			OSS (0.527 μg/insect)			OSS-R (0.527 μg/insect)		
	Unpen.	Inter.	Excre.	Unpen.	Inter.	Excre.	Unpen.	Inter.	Excre.
I	96.7	15.2	7.6	97.0	7.3	80.7	97.3	6.6	52.8
II		2.0	1.5		2.1	1.0		0.8	0.6
III		9.2	4.0		8.3	1.2		1.8	1.1
IV		12.8	5.6		18.1	3.2		13.4	9.1
V		7.0	7.0		10.2	4.3		13.2	2.0
VI		11.7	10.8		13.7	2.6		11.7	5.8
VII		3.7	3.9		8.9	4.4		5.5	1.2
VIII		13.6	33.8		8.0	1.2		29.7	19.2
IX		6.4	15.0		13.4	0.7		5.5	3.8
X		9.1	6.7		8.5	0.5		6.8	3.4
XI		9.3	4.1		1.5	0.2		5.0	1.0
Total D.M.[b]	3.3	82.8	90.9	3.0	90.8	18.3	2.7	92.6	46.6
% RR[c]	38.3	26.3	35.4	61.3	30.1	8.6	58.7	9.9	31.4

[a] Met.: Metabolite.
[b] Total D.M.: Total degradative metabolites.
[c] % RR: Percentage of radioactivity recovered (values for internal and excreted quantities express the radioactivity extracted by methanol).

Table 8 Metabolites recovered from OKR and OKR-R strains of the diamondback moth 24 hr after topical application of [methoxy-^{14}C]phenthoate (LD_{10} and 1.02 μg/insect).

Met.[a]	Percentage of radioactivity								
	OKR (0.038 μg/insect)			OKR (1.02 μg/insect)			OKR-R (1.02 μg/insect)		
	Unpen.	Inter.	Excre.	Unpen.	Inter.	Excre.	Unpen.	Inter.	Excre.
I	92.5	11.7	13.8	97.1	12.5	78.3	95.6	11.5	52.1
II		2.0	1.2		3.0	1.0		1.0	0.3
III		4.5	2.5		8.0	1.6		3.6	1.2
IV		9.9	6.6		23.3	3.1		28.9	11.4
V		11.8	4.5		12.1	2.9		7.2	5.1
VI		17.1	8.9		13.1	2.7		13.8	5.2
VII		4.5	5.3		9.5	3.7		5.4	0.9
VIII		13.5	33.3		11.2	4.4		11.3	16.7
IX		12.4	15.8		3.5	0.9		8.0	2.7
X		6.3	4.1		2.2	1.1		5.8	3.3
XI		6.3	4.0		1.7	0.3		3.5	1.1
Total D.M.[b]	7.5	86.3	85.0	2.9	84.5	20.7	4.4	87.5	47.6
% RR[c]	35.8	23.3	40.9	73.2	20.2	6.6	47.5	10.9	41.6

[a], [b] and [c]: as in Table 7.

showed that the resistant strains produced larger amounts of compound **IV**, while the susceptible strains yielded larger amounts of compound **VIII** (Tables 8–10).

Phenthoate metabolism in the diamondback moth appears to be similar to that in plants,[13,19] white mouse and housefly.[16] It is clear that the diamondback moth is capable of degrading phenthoate almost equally through hydrolysis of the carboethoxy moiety, cleavage of the P–

Table 9 Metabolites recovered from OSS and OSS-R strains of the diamondback moth 24 hr after topical application of [methoxy-^{14}C]phenthoate (LD_{50}).

Met.[a]	Percentage of radioactivity					
	OSS (0.102 μg/insect)			OSS-R (15.60 μg/insect)		
	Unpen.	Inter.	Excre.	Unpen.	Inter.	Excre.
I	92.9	19.6	32.4	97.6	19.5	71.0
II		1.0	0.4		2.4	1.0
III		2.0	2.2		5.4	1.1
IV		11.7	6.2		22.3	5.6
V		5.0	2.9		4.6	2.8
VI		15.4	10.4		15.3	2.2
VII		5.8	1.8		1.6	0.8
VIII		24.8	36.3		10.5	5.4
IX		4.9	1.7		7.8	5.0
X		5.2	4.2		5.4	2.9
XI		4.6	1.5		5.2	2.2
Total D.M.[b]	7.1	79.4	67.2	2.4	78.1	28.0
% RR[c]	24.4	34.3	41.3	62.3	15.9	21.8

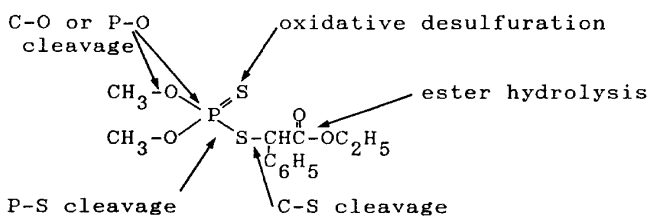
[a], [b] and [c]: as in Table 7.

Table 10 Metabolites recovered from OKR and OKR-R strains of the diamondback moth 24 hr after topical application of [methoxy-^{14}C]phenthoate (LD_{50}).

Met.[a]	Percentage of radioactivity					
	OKR (0.160 μg/insect)			OKR-R (32.64 μg/insect)		
	Unpen.	Inter.	Excre.	Unpen.	Inter.	Excre.
I	95.5	17.0	53.8	97.9	19.7	85.7
II		0.6	0.7		1.0	0.5
III		0.7	0.8		5.5	0.6
IV		20.6	5.2		23.6	3.4
V		7.4	1.7		10.8	1.9
VI		18.9	6.6		11.6	1.4
VII		1.2	1.1		2.4	0.4
VIII		19.5	23.3		10.2	3.8
IX		5.5	2.5		5.2	0.9
X		5.1	3.3		4.1	1.0
XI		3.5	1.0		5.9	0.7
Total D.M.[b]	4.5	82.4	45.5	2.1	79.3	13.8
% RR[c]	31.8	41.1	27.1	53.6	7.9	38.5

[a], [b] and [c]: as in Table 7.

S or C–S bond, and removal of the methoxy group by either direct demethylation or hydrolytic cleavage of the P–O bond. Based on the amount of oxon metabolites such as **II** and **VI**, it is possible to assume that a significant portion of administered phenthoate was metabolized to phenthoate oxon (**II**).



Comparison of the data on phenthoate

metabolism in the resistant and susceptible strains of the diamondback moth (Tables 7–10) reveals that hydrolysis of the carboethoxy moiety and C–S cleavage plays an important role in the resistant strains than in the susceptible strains (compare metabolite **IV**). On the other hand, the susceptible strains show greater hydrolysis of the carboethoxy moiety of demethyl phenthoate (**III**) than the resistant strains. The results obtained here seem to show that there are no significant differences in phenthoate metabolism between the susceptible and resistant strains. Even though there are some differences in amounts of compound **IV**, it is doubtful that these differences are significant.

In summary, it is evident that increased phenthoate metabolism is not a major factor for the resistance of the OSS-R and OKR-R strains, but reduced rate of penetration is more likely an important factor for the phenthoate resistance in the diamondback moth. It is possible to postulate how resistance develops: decreased penetration results in slower and less internal accumulation of toxicant. The normal rate of metabolism prevents the levels of toxicant from reaching a higher threshold; thus, a toxic response does not occur.

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要　　約

抵抗性および感受性コナガのフェントエートの表皮透過性と代謝

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[Methoxy-^{14}C]phenthoate を用い，コナガ幼虫での表皮透過性および *in vivo* での代謝を調べた．抵抗性コナガ幼虫では，感受性系統よりも [methoxy-^{14}C]phenthoate の表皮透過性が低く，体内存在量も少なく，体外排泄率が高かった．*In vivo* における代謝割合は抵抗性系統と感受性系統とで差はなかった．また，代謝物として ^{14}C-炭酸ガスはみられなかった．以上のことから，コナガにおけるフェントエート抵抗性機構として，抵抗性系統におけるフェントエートの表皮透過性の減少が重要と考えられた．