Original Article

Structure-resistance Relationship in the Organophosphorus-resistant Rice Stem Borer, *Chilo suppressalis*

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Relationships between the chemical structure of 61 organophosphorus (OP) insecticides and the degree of resistance in the OP-resistant rice stem borer, *Chilo suppressalis* WALKER, were studied. The resistant strain necessarily showed high levels of resistance to various types of OP insecticides containing the aryl (including the aromatic heterocyclic group) ester bond, such as fenitrothion, cyanophos, pirimiphos-methyl, chlorpyrifos-methyl, pirimiphos-ethyl, EPN, isofenphos, and their oxons. Resistance was higher to dimethyl esters than to their diethyl homologs, and the highest resistance ratios were found with heterocyclic esters. In contrast, essentially no resistance to OP insecticides having the ester bond with aliphatic enol and thioalcohol, such as malathion, phenthoate, azinphos-methyl, malaoxon, tetrachlorvinphos, and monocrotophos was observed. It is, therefore, concluded that a P-O-aryl bond in OP insecticides is prerequisite for the resistance.

INTRODUCTION

The rice stem borer, *Chilo suppressalis* WALKER, is an important pest for rice plants in Asia. In 1960, a resistant strain to parathion was first recorded in Kagawa prefecture.¹⁾ In 1978, there was a sudden outbreak of the rice stem borer in Okayama prefecture and it became apparent that the rice stem borer showed high resistance to several organophosphorus (OP) insecticides such as fenitrothion, fenthion, diazinon, propaphos and tri-chlorfon.²⁻⁴⁾

We attempted to clarify the mechanism of resistance in the OP-resistant Okayama strain of the rice stem borer. A preliminary report showed that the increased detoxication of fenitrothion in the resistant strain is a major factor in the mechanism of resistance to fenitrothion.⁵⁾ This paper deals with the relationship between the chemical structure of 61 OP insecticides and the degree of resistance

in the OP-resistant rice stem borer.

MATERIALS AND METHODS

1. Insect

The OP insecticide resistant (R) strain of the rice stem borer was collected at Hata in Soja, Okayama prefecture in 1983 and maintained for several generations in the laboratory without insecticidal selection pressure. The susceptible (S) strain was supplied by the Institute of Physical and Chemical Research, and was maintained in the laboratory. Larvae of 60 to 70 mg body weight of the R and S strains were used for this study.

2. Toxicity Evaluation

One-half microliter of acetone solution of insecticides was applied topically with a microapplicator to the dorsal part of each larva's abdomen. Each insecticide was tested at each dosage level with triplicates of ten larvae. Treated larvae were kept at 26°C.

Mortality was counted 24 hr after treatment. LD₅₀ values were determined from computed log dose probit lines.

3. Chemicals

Table 1 lists common names and chemical formula of 61 OP insecticides tested. Samples of insecticides were synthesized by published procedures,⁶⁻¹¹⁾ extracted from commercial emulsifiable concentrates, or provided by the Institute of Physical and Chemical Research

and manufacturers.

RESULTS

Sixty-one OP insecticides were examined for toxicity to both the R and S strains of the rice stem borer and the degree of resistance was measured by the resistance factor (R/S). Tables 2 and 3 summarize results of tests with *O,O*-dialkyl *O*-aryl (including the aromatic heterocyclic group) phosphorothioates and phosphates, respectively. The R strain showed

Table 1 List of OP insecticides used.

Common name	Chemical formula or chemical name
Fenitrothion	(MeO) ₂ (3-Me-4-NO ₂ PhO)PS
Parathion-methyl	(MeO) ₂ (4-NO ₂ PhO)PS
Dicapthion	(MeO) ₂ (2-Cl-4-NO ₂ PhO)PS
Chlorthion	(MeO) ₂ (3-Cl-4-NO ₂ PhO)PS
Cyanophos	(MeO) ₂ (4-CNPhO)PS
Fenthion	(MeO) ₂ (3-Me-4-MeSPhO)PS
Pirimiphos-methyl	(MeO) ₂ (2-Me ₂ N-6-Me-4-pyrimidinylO)PS
Methyl isoxathion	(MeO) ₂ (5-Ph-3-oxazolylO)PS
Methyl pyridaphenthion	(MeO) ₂ (2,3-dihydro-3-oxo-2-Ph-6-pyridazinylO)PS
Chlorpyrifos-methyl	(MeO) ₂ (3,5,6-Cl ₃ -2-pyridylO)PS
Methyl diazinon	(MeO) ₂ (2-iso-Pr-6-Me-4-pyrimidinylO)PS
Ethyl fenitrothion	(EtO) ₂ (3-Me-4-NO ₂ PhO)PS
Parathion	(EtO) ₂ (4-NO ₂ PhO)PS
Ethyl dicapthion	(EtO) ₂ (2-Cl-4-NO ₂ PhO)PS
Ethyl chlorthion	(EtO) ₂ (3-Cl-4-NO ₂ PhO)PS
Pirimiphos-ethyl	(EtO) ₂ (2-Me ₂ N-6-Me-4-pyrimidinylO)PS
Isoxathion	(EtO) ₂ (5-Ph-3-oxazolylO)PS
Pyridaphenthion	(EtO) ₂ (2,3-dihydro-3-oxo-2-Ph-6-pyridazinylO)PS
Chlorpyrifos	(EtO) ₂ (3,5,6-Cl ₃ -2-pyridylO)PS
Diazinon	(EtO) ₂ (2-iso-Pr-6-Me-4-pyrimidinylO)PS
Fenitroxon	(MeO) ₂ (3-Me-4-NO ₂ PhO)PO
Methyl paraoxon	(MeO) ₂ (4-NO ₂ PhO)PO
Dicapthion-oxon	(MeO) ₂ (2-Cl-4-NO ₂ PhO)PO
Chlorthion-oxon	(MeO) ₂ (3-Cl-4-NO ₂ PhO)PO
Fenthion-oxon	(MeO) ₂ (3-Me-4-MeSPhO)PO
Pirimiphos-methyl-oxon	(MeO) ₂ (2-Me ₂ N-6-Me-4-pyrimidinylO)PO
Methyl isoxathion-oxon	(MeO) ₂ (5-Ph-3-oxazolylO)PO
Methyl pyridaphenthion-oxon	(MeO) ₂ (2,3-dihydro-3-oxo-2-Ph-6-pyridazinylO)PO
Chlorpyrifos-methyl-oxon	(MeO) ₂ (3,5,6-Cl ₃ -2-pyridylO)PO
Methyl diazoxon	(MeO) ₂ (2-iso-Pr-6-Me-4-pyrimidinylO)PO
Ethyl fenitroxon	(EtO) ₂ (3-Me-4-NO ₂ PhO)PO
Paraoxon	(EtO) ₂ (4-NO ₂ PhO)PO
Ethyl dicapthion-oxon	(EtO) ₂ (2-Cl-4-NO ₂ PhO)PO
Pirimiphos-ethyl-oxon	(EtO) ₂ (2-Me ₂ N-6-Me-4-pyrimidinylO)PO
Isoxathion-oxon	(EtO) ₂ (5-Ph-3-oxazolylO)PO
Pyridaphenthion-oxon	(EtO) ₂ (2,3-dihydro-3-oxo-2-Ph-6-pyridazinylO)PO
Chlorpyrifos-oxon	(EtO) ₂ (3,5,6-Cl ₃ -2-pyridylO)PO

(to be continued)

Table 1 (Continued).

Common name	Chemical formula or chemical name
Diazoxon	(EtO) ₂ (2-iso-Pr-6-Me-4-pyrimidinylO)PO
Propaphos	(PrO) ₂ (4-MeSPhO)PO
<i>n</i> -Propyl paraoxon	(PrO) ₂ (4-NO ₂ PhO)PO
Isopropyl paraoxon	(iso-PrO) ₂ (4-NO ₂ PhO)PO
Dimethylvinphos	(MeO) ₂ PO[OC(=CHCl)PhCl ₂ -2,4]
Tetrachlorvinphos	(MeO) ₂ PO[OC(=CHCl)PhCl ₃ -2,4,5]
Monocrotophos	(MeO) ₂ PO(OCMe=CHCONHMe)
Dichlorvos	(MeO) ₂ PO(OCH=CCl ₂)
Malathion	(MeO) ₂ PS[Sch(CH ₂ COOEt)COOEt]
Thiometon	(MeO) ₂ PS(SCH ₂ CH ₂ SEt)
Dimethoate	(MeO) ₂ PS(SCH ₂ CONHMe)
Phenthoate	(MeO) ₂ PS[Sch(COOEt)Ph]
Azinphos-methyl	(MeO) ₂ (3,4-dihydro-4-oxobenzo[<i>d</i>]-1,2,3-triazixin-3-yl-methylS)PS
Prothiophos	(EtO)(PrS)(2,4-Cl ₂ PhO)PS
Malaoxon	(MeO) ₂ PO[Sch(CH ₂ COOEt)COOEt]
EPN	(EtO)(4-NO ₂ PhO)PS(Ph)
EPN-oxon	(EtO)(4-NO ₂ PhO)PO(Ph)
Cyanofenphos	(EtO)(4-CNPhO)PO(Ph)
Trichlorfon	(MeO) ₂ PO[CH(OH)CCl ₃]
Isofenphos	(EtO)(2-Me ₂ CHOCOPhO)PS(NHPr-iso)
Acephate	(MeO)(MeS)PO(NHCOMe)
Salithion	2-Methoxy-benzo-4 <i>H</i> -1,3,2-dioxaphosphorixin-2-sulfide
TEPP	(EtO) ₂ P(O)OP(O)(OEt) ₂
Sulfotep	(EtO) ₂ P(S)OP(S)(OEt) ₂

Table 2 Toxicity of *O,O*-dialkyl *O*-aryl phosphorothioates to resistant and susceptible rice stem borer.

Insecticides	LD ₅₀ (μg/g)		Resistance factor (R/S)
	Susceptible (S)	Resistant (R)	
Fenitrothion	1.8	75.3	41.8
Parathion-methyl	0.9	18.9	21.0
Dicapthon	15.6	277.6	17.8
Chlorthion	12.4	334.5	27.0
Cyanophos	1.3	73.3	56.4
Fenthion	3.1	81.6	26.3
Pirimiphos-methyl	2.4	710.1	295.9
Methyl isoxathion	1.5	131.3	87.5
Methyl pyridaphenthion	11.3	970.7	85.9
Chlorpyrifos-methyl	1.3	94.0	72.3
Methyl diazinon	4.0	124.0	31.0
Ethyl fenitrothion	10.1	60.7	6.0
Parathion	2.6	49.6	19.1
Ethyl dicapthon	27.6	235.8	8.5
Ethyl chlorthion	29.1	215.7	7.4
Pirimiphos-ethyl	2.0	150.9	75.5
Isoxathion	8.0	118.8	14.9
Pyridaphenthion	1.9	60.5	31.8
Chlorpyrifos	1.3	67.5	51.9
Diazinon	6.2	125.6	20.3

Table 3 Toxicity of *O,O*-dialkyl *O*-aryl phosphates to resistant and susceptible rice stem borer.

Insecticides	LD ₅₀ (μg/g)		Resistance factor (R/S)
	Susceptible (S)	Resistant (R)	
Fenitroxon	1.2	41.3	34.4
Methyl paraoxon	0.5	7.2	14.4
Dicaphthion-oxon	4.1	85.6	20.9
Chlorthion-oxon	6.4	105.7	16.5
Fenthion-oxon	3.0	30.1	10.0
Pirimiphos-methyl-oxon	0.3	29.9	99.7
Methyl isoxathion-oxon	1.0	22.1	22.1
Methyl pyridaphenthion-oxon	9.7	188.2	19.4
Chlorpyrifos-methyl-oxon	0.4	16.8	42.0
Methyl diazoxon	1.3	15.7	12.1
Ethyl fenitroxon	7.8	55.5	7.1
Paraoxon	5.2	35.1	6.8
Ethyl dicaphthion-oxon	4.8	48.2	10.0
Pirimiphos-ethyl-oxon	2.1	30.8	14.7
Isioxathion-oxon	1.5	13.8	9.2
Pyridaphenthion-oxon	2.3	27.1	11.8
Chlorpyrifos-oxon	0.6	23.7	39.5
Diazoxon	3.2	17.1	5.3
Propaphos	8.2	61.5	7.5
<i>n</i> -Propyl paraoxon	5.7	136.7	24.0
Isopropyl paraoxon	605.7	>5000.0	>8.3

resistance to all insecticides tested. Of the 20 phosphorothioates, the R strain was very resistant to pirimiphos-methyl (295×), and was 40–90 times more resistant to methyl isoxathion, methyl pyridaphenthion, pirimiphos-ethyl, chlorpyrifos-methyl, cyanophos, chlorpyrifos and fenitrothion than the S strain. With the phosphate insecticides, relatively high resistance was demonstrated to pirimiphos-methyl-oxon, chlorpyrifos-methyl-oxon, chlorpyrifos-oxon and fenitroxon. The resistance factor generally was lower for the oxygen analogs than for their corresponding phosphorothioates. The phosphorothioates and phosphates showed a trend toward decreasing the resistance factor with an increase in the carbon number of the dialkyl groups. Resistance was generally higher in the heterocyclic esters than the phenolic esters, especially in the dimethyl compounds. The effect of the nature and position of aryl substituents on the resistance factor did not show a consistent pattern.

Table 4 summarizes results of tests with 4

Table 4 Toxicity of *O,O*-dimethyl *O*-vinyl phosphates to resistant and susceptible rice stem borer.

Insecticides	LD ₅₀ (μg/g)		Resistance factor (R/S)
	Susceptible (S)	Resistant (R)	
Dimethylvinphos	0.3	0.6	2.0
Tetrachlorvinphos	2.1	2.2	1.0
Monocrotophos	2.2	6.9	3.1
Dichlorvos	24.6	486.8	19.8

phosphate insecticides having aliphatic enol ester structure. Resistance disappeared in aliphatic enol ester phosphates except for dichlorvos.

LD₅₀ values of the phosphorodithioates and phosphorothiolates for the two strains are given in Table 5. No resistance was demonstrated in the R strain to OP insecticides having the ester bond with aliphatic thioalcohol such as malathion, thiometon, dimethoate, phenthoate, azinphos-methyl and

Table 5 Toxicity of phosphorodithioates and phosphorothiolate to resistant and susceptible rice stem borer.

Insecticides	LD ₅₀ (μg/g)		Resistance factor (R/S)
	Susceptible (S)	Resistant (R)	
Malathion	1.5	1.7	1.1
Phenthoate	3.1	2.3	0.7
Thiometon	87.8	101.7	1.2
Dimethoate	13.3	18.5	1.4
Azinphos-methyl	0.9	1.2	1.3
Prothiophos	20.1	187.1	9.3
Malaoxon	5.8	8.2	1.4

malaoxon. In contrast, the R strain was resistant to prothiophos which is the dithioester containing a *P-O*-aryl ester bond.

High resistance factor was found for aryl phosphonothioate and phosphonate insecticides as well as aryl phosphorothioate and phosphate insecticides (Table 6). In addition, as reported previously,³⁾ the R strain showed high resistance to trichlorfon, a precursor of dichlorvos.

For a better understanding of the structure-resistance relationship in the OP-resistant rice stem borer, OP insecticides of the type (R) (R')P(S)(R'') where R, R' and R'' are various combinations of alkoxy, alkylthio, amido, and phenyl moieties were examined (Table 7). The R strain showed high resistance to the aryl phosphoramidothioate, isofenphos; slight resistance to the saligenin cyclic ester, salithion; and no cross resistance to the aliphatic phosphoramidothiolate, acephate. Here again resistance was shown in the R strain by OP insecticides having the *P-O*-aryl bond.

With pyro ester insecticides, resistance was demonstrated in the R strain to TEPP and sulfotep.

DISCUSSION

It was demonstrated in this study that the R strain of the rice stem borer collected in Okayama prefecture was without exception resistant to OP insecticides with the ester linkage to an aromatic carbon among 61 OP insecticides. In contrast, the R strain generally was susceptible to OP insecticides with

Table 6 Toxicity of phosphonothioates and phosphonates to resistant and susceptible rice stem borer.

Insecticides	LD ₅₀ (μg/g)		Resistance factor (R/S)
	Susceptible (S)	Resistant (R)	
EPN	0.7	22.7	32.4
EPN-oxon	1.0	21.6	21.6
Cyanofenphos	6.4	130.0	20.3
Trichlorfon	7.6	591.9	77.9

Table 7 Toxicity of various OP insecticides to resistant and susceptible rice stem borer.

Insecticides	LD ₅₀ (μg/g)		Resistance factor (R/S)
	Susceptible (S)	Resistant (R)	
Isofenphos	3.5	151.2	43.2
Acephate	70.0	103.4	1.5
Salithion	2.5	16.3	6.5
TEPP	24.6	281.1	11.4
Sulfotep	44.8	960.4	21.4

the ester linkage to an aliphatic carbon, although there were exceptions. It is, therefore, concluded that the ester linkage to an aromatic carbon in OP insecticides is very important for the resistance.

Conversion of a phosphorothioate, or P=S compound, to a phosphate, or P=O compound, is generally known as the most common activation in the insect body. In this investigation, the R strain showed cross resistance to all *O,O*-dialkyl *O*-aryl phosphates and phosphonates corresponding to the P=S compounds (Tables 3 and 6). It is, therefore, possible to speculate that the resistance mechanism to various aryl ester insecticides is closely associated with their oxon analogs. In fact, our preliminary report indicated that an increased degradation of fenitroxon to non-toxic water-soluble metabolites in the R strain is the major mechanism of the resistance to fenitrothion.⁵⁾

In Okayama prefecture, fenitrothion, fenthion and diazinon have been primarily used for the control of the rice stem borer since the late 1960's.³⁾ This continuous use of

these insecticides may lead to the development of high levels of resistance to various aryl ester insecticides. The resistance mechanism of the R strain differs from that of the parathion-resistant strain found in Kagawa prefecture in 1960, since no cross resistance to fenitrothion and parathion-methyl was demonstrated in the parathion-resistant strain.¹⁾

The R strain also showed resistance to trichlorfon and dichlorvos. Since trichlorfon and other aliphatic insecticides have been used to a limited extent for control of the rice stem borer, the reason for causing the high resistance is uncertain.

Susceptibility and cross resistance to various OP insecticides have been examined in the diazinon-selected SKA strain of the housefly¹²⁾ and the malathion- and parathion-methyl-selected strains of the green rice leafhopper¹³⁾ in connection with their resistance mechanism. In the housefly, although resistance was higher in the ethyl than in the corresponding methyl compounds, the structure-resistance relationship was not so clear as that in the OP-resistant rice stem borer. In the green rice leafhopper, resistance was shown consistently to all OP insecticides examined regardless of the nature of their ester bond. Thus it seems probable that resistance mechanism(s) in the OP-resistant rice stem borer is simpler than those in the housefly¹⁴⁾ and the green rice leafhopper.¹³⁾

These results of the structure-resistance study may provide useful information on the resistance mechanism and on the design of the most potent insecticides which overcome the resistance of the rice stem borer.

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Ltd., Tokyo; dimethylvinphos and tetrachlorvinphos from Kumiai Chemical Industry Co., Ltd., Tokyo; diazinon and propaphos from Nippon Kayaku Co., Ltd., Tokyo; monocrotophos from Nihon Nohyaku Co., Ltd., Tokyo; and fenthion from Nihon Tokushu Noyaku Seizo K. K., Tokyo.

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要 約

有機リン剤抵抗性ニカメイガにおける化学構造と抵抗性の相関

昆野安彦, 宍戸 孝, 田中福三郎

有機リン剤に対して抵抗性の発達した岡山県総社市秦産ニカメイガ(R系統)の抵抗性機構を明らかにするために61種の有機リン殺虫剤を用いてその殺虫活性感受性(S)系統と比較し, 化学構造と抵抗性の相関について検討した。R系統は, 芳香環または芳香族複素環エステル結合をもつphosphorothioate, phosphorodithioate, phosphonothioate, phosphoramidodithioate, およびそれらのオクソン等に対して例外なく抵抗性を示した。ジメチル型はジエチル型よりも抵抗性比(R/S)が高く, とくに芳香族複素環をもつ有機リン殺虫剤に対して高度の抵

抗性を示すことが判明した（ピリミホスメチル，295.9 倍；メチルイソキサチオン，87.5 倍）。一方，脂肪族エノールやチオアルコールとエステル結合を形成する脂肪族系有機リン殺虫剤に対しては，一般に抵抗性の発達は

認められなかった。以上の結果，エステルの芳香族性が岡山産ニカメイガの抵抗性発現への重要な条件であることが明らかになった。