strain.

For both strains, most of P=O compounds tested showed low LD_{50} values in comparison with P=S compounds, and P=O compounds showed much lower LD_{50} values than their P=S analogues. These facts suggest that the detoxification before oxidation or activation of P=S compounds to P=O is an important mechanism in the organophosphorus-resistance of the green rice leafhopper.

The two resistant strains showed no cross resistance to both organochlorine and carbamate insecticides.

Mechanism of Resistance to Malathion in the Green Rice Leafhopper, *Nephotettix cincticeps* Uhler. Hiroshi HAMA, Toshikazu IWATA, Chōjirō TOMIZAWA and Toshinobu MURAI (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo, Japan) Received Sept. 9, 1977. *Botyu-Kagaku*, 42, 188, 1977.

29. ツマグロヨコバイにおけるマラソン抵抗性の機構

浜 弘司，岩田俊一，富沢長次郎，村井敏信（農林省農業技術研究所，東京都北区西ケ原2-1） 52. 9. 9 受理

ツマグロヨコバイの有機リン剤抵抗性の2系統（MとP）と感受性系統（S）を用い，共力剤の効果，マラソンの浸透と代謝およびコリンエステラーゼの感受性を測定，比較し本種のマラソン抵抗性の機構について考察した．マラソンの浸透およびコリンエステラーゼの感受性では系統間の違いが認められなかった．マラソンはいずれの系統でも *in vivo, in vitro* で速やかに水溶性代謝物に分解されたが，M，P両抵抗性系統の代謝活性はS系統より2倍程度高かった．主な代謝物はマラソンの monoacid であった．他の未同定の代謝物も検出され，それらは経時的に増大したが，水溶性代謝物のイオン交換クロマトグラムでは系統間の顕著な違いはみられなかった．一方，体内のトルエン可溶分画は大部分が未分解のマラソンであったが，その酸化物であるマラオクソンはS系統のみで検出されM，P両抵抗性系統では検出されなかった．したがって，M，P両系統のマラソン抵抗性はマラオクソンの蓄積が極めて小さいことで説明された．さらにマラオクソンの蓄積に影響を及ぼす因子について論及した．

Introduction

Resistance to malathion in insects such as the housefly has been extensively studied and its main mechanism was proved to be the increased activity of carboxylesterase hydrolyzing carboxylic esters of malathion to nontoxic acid metabolites. It has been demonstrated in *Musca domestica*[1,2], *Culex tarsalis*[3-5], *Chrysomya putoria*[6] and *Tribolium castaneum*[7] that carboxylesterase activity is higher in malathion-resistant strains than in susceptible ones and that inhibitors of carboxylesterase exhibit synergistic effect on toxicity of malathion for the resistant strains. Moreover, the malathion-resistant strains of these insects show little cross-resistance to the other organophosphates having no carboxylic ester[8].

It has been observed that malathion-resistance is also developed by selection with the other organophosphates in *Musca domestica*[9] and *Blattella germanica*[10]. Such a resistance appeares to be attributed to another factor different from carboxylesterase.

Kojima *et al.*[11] showed that in the green rice leafhopper *in vitro* degradation rate of malathion was higher in a malathion-resistant strain than in a susceptible one and that naled (Dibrom), an inhibitor of carboxylesterase, exhibited synergistic effect on the toxicity of malathion. They concluded that the malathion-resistance in this leafhopper was attributed to the increased activity of carboxylesterase. On the other hand, it was shown that nonspecific aliesterase (AliE) activity hydrolyzing many simple aliphatic and aromatic esters[12] was very high in the resistant leafhopper as compared with that in the susceptible one[11,13-16] and was linked with the resistance to malathion[17]. These findings were followed by the report[18], in which an esterase activity hydrolyzing carbophenoxy malathion 〔*O, O*-dimethyl *S*-(1, 2-bis-carbophenoxy)ethyl phosphorodithioate〕 was high in the resistant leafhopper, and was inhibited by a saligenin cyclic phenyl phosphate K2, a

synergist to malathion. More recently, Miyata and Saito[19] investigated relationships between AliE and carboxylesterase in this leafhopper and discussed the same subject as presented in this paper.

Results cited above support the conclusion proposed previously by Kojima *et al.*[11] However, most of the resistant leafhoppers recently collected from fields show resistance not only to malathion at high level but also to many other organophosphates having no carboxylic ester[20,21]. Such resistance patterns in this leafhopper seem to be a result of selection with various insecticides used for control of this leafhopper or other pests. It is also likely that most of resistant populations has developed a high level of resistance to malathion as a result of selection with both malathion and the other organophosphates.

The present authors investigated effects of some synergists on toxicity of malathion and malaoxon, penetration and metabolism of malathion, and sensitivity of cholinesterase (ChE) to malathion and malaoxon in order to clarify mechanism of the malathion-resistance in the green rice leafhopper which developed high level of resistance to various organophosphates.

Materials and Methods

Insect: Two organophosphate-resistant strains (M and P) and one susceptible strain (S) of the green rice leafhopper, *Nephotettix cincticeps* Uhler, were used[22]. Both M and P strains are derived from an organophosphate-resistant population collected at Doi in Ehime prefecture in 1965. Doi population was divided into two groups, and selected with malathion or methyl parathion in this laboratory. M strain selected with malathion shows 380 times resistance to malathion and P strain selected with methyl parathion shows 79 times resistance to malathion as compared with S strain[22]. The two resistant strains show also extremely high level of resistance to many other organophosphates but no cross-resistance to carbamate insecticides[22]. S strain was collected in Miyagi prefecture in 1969 and has been used as a standard strain. This strain is high susceptible to many organophosphates and carbamates[22]. For all experiments, females 4 to 9 days after emergence were used.

Insecticide and synergist: Malaoxon was obtained by oxidation of malathion with bromine-water. Malathion (96%) and malaoxon thus obtained were purified by thin-layer chromatography (TLC) on a silica gel plate with the solvent system of hexane and acetone (19: 1). Tri-*o*-cresyl phosphate (TOCP), triphenyl phosphate (TPP) and piperonyl butoxide were purchased from Tokyo Kasei Kogyo Co. Ltd. EPN-oxon and isopropyl paraoxon were kindly supplied by Drs. T. Shishido and J. Fukami.

Synthesis of radio-labeled malathion: ^{32}P, ^{35}S-double labeled malathion was synthesized according to the following equations as reported by

Casida[23] and March *et al.*[24]:

$$P_2S_5 \xrightarrow{H_3{}^{32}PO_4} {}^{32}P_2S_5$$

$$P + {}^{35}S \rightarrow {}^{32}P_2{}^{35}S_3 \xrightarrow{CH_3OH} (CH_3O)_2{}^{32}P(={}^{35}S)SH$$

$$CHCOOC_2H_5 \quad {}^{35}S$$
$$\vert \qquad \vert$$
$$CHCOOC_2H_5 \rightarrow (CH_3O)_2{}^{32}P(=O)SCHCOOC_2H_5$$
$$\qquad\qquad\qquad\qquad\quad \vert$$
$$\qquad\qquad\qquad\qquad\quad CH_2COOC_2H_5$$

The labeled malathion was purified by TLC as mentioned above, and its radiochemical purity was more than 99% for ^{32}P and ^{35}S, and specific activity was approximately 1mC/mM for ^{32}P and 3mC/mM for ^{35}S at the beginning of the experiment.

Synthesis of metabolites of malathion: *O*, *O*-Dimethyl hydrogen phosphorodithioate, malathion diacid [*O*, *O*-dimethyl *S*-(1, 2-bis-carboxy) ethyl phosphorodithioate] and malathion monoacid [*O*, *O*-dimethyl *S*-(1-carboxy-2-carboethoxy) ethyl phosphorodithioate] were synthesized according to the method described by March *et al.*[24] *O*, *O*-Dimethyl hydrogen phosphorothioate was synthesized based on the method given by Foss[25].

Toxicity test: Topical application technique[22] was adopted for test of synergists. In simultaneous application method, an insecticide and a synergist in acetone were mixed with an appropriate ratio and 0.5μl of the mixture was applied on the dorsal surface of the adult anesthetized with carbon dioxide. In pre-application method, 0.5μl of acetone solution of a synergist was applied 60 min before the application of acetone solution (0.5μl) of an insecticide. The treated insects were placed in a plastic case containing the rice seedlings and kept at 27±1.5°C. Mor-

tality was counted 24 hr after treatment.

Penetration and metabolism of malathion: A 0.1μl-drop of acetone solution of ^{32}P, ^{35}S-labeled malathion was applied topically on the dorsal surface of the adult anesthetized with carbon dioxide. Two dosages were applied:0.1μg at the low dosage and 1.0μg at the high dosage. The treated insects were placed in a plastic case containing the rice seedlings and kept at 27±1.5° C. Symptom of the treated insects was observed at various time intervals and then distribution of radioactivity in external and internal of the insect body was determined.

The treated insects(70-100 adults for one group) were rinsed on a funnel with 10ml of toluene and 1ml of the toluene solution was taken for radioassay. The activity in toluene was referred to as the external. The rinsed insects were homogenized in cold 0.2% trichloroacetic acid(TCA) solution (below pH 2) and the homogenates were centrifuged at 700g for 5 min to remove residue. The residues were again washed with the TCA solution. The residues were subjected to combustion by nitric acid, followed by perchloric acid. Resultant solutions were neutralized with KOH solution and aliquots were taken into vials for radioassay. The supernatants were combined and adjusted to pH 7.0 with 0.5 N KOH. Then, the equal volume of toluene was added to the solution and shaken vigorously. After centrifugation of the solution, 1ml from toluene fractions and 2ml from aqueous fractions were taken into vials and 10ml of toluene-based scintillation fluid (PPO, 3.75g and POPOP, 0.15g/l) and dioxane-based scintillation fluid(PPO, 0.15g and POPOP, 0.15g and naphthalane, 45g/l) were added to respective vials. Measurement of radioactivity was conducted by Tricarb liquid scintillation spectrometer (model 3003).

Remained portions of the toluene fractions were concentrated to a small volume and the concentrate was chromatographed on silica gel plates with the solvent system of hexane and acetone (19:1). Radioautograms of the TLC plates were prepared by the conventional method. Malathion and malaoxon on the plate were identified by co-chromatography with the authentic compounds. Radioactive portions on the plate were scraped and their radioactivities were determined.

The aqueous fractions were subjected to an ion-exchange chromatography on Dowex 1-X8 and gradient elution with ascending concentrations of hydrochloric acid[26] was conducted as shown in Fig. 3. Monoacid and diacid of malathion were identified by co-chromatography with the authentic compounds. Although other metabolites could not be identified, *O*, *O*-dimethyl hydrogen phosphorothioate and *O*,*O*-dimethyl hydrogen phosphorodithioate were confirmed to be eluted in gradient III and IV, respectively.

In vitro degradation of malathion: Females were homogenized in 1/15 M phosphate buffer pH 7.2 (20 adults/ml) in a glass homogenizer. The homogenate was centrifuged at 20,000g for 20 min. The supernatant liquid and the crude homogenate were used as ane nzyme source. Four ml of the enzyme solution equivalent to 80 females and 20μg of malathion dissolved in 10μl of ethanol were taken in a test tube, and incubated at 30°C for 2 hr. Malathion in the incubation mixture was extracted 3 times with 4ml of hexane. The extracts were concentrated to 1 or 2ml and malathion was determined by gas chromatography. Operational conditions of gas chromatography were as follows: apparatus, Shimadzu GC-5A; detector, flame photometric detector (P filter); column, pyrex column (3mmϕ×1m) packed with 10% Silicone DC-200 coated on 60-80 mesh Gas-chromQ; column temperature, 205°C; flow rate of carrier gas (N_2), 80ml/min.

Sensitivity of ChE to malathion and malaoxon: Females were homogenized in 1/15 M phosphate buffer pH 7.2 (10 adults/ml). The homogenate was centrifuged at 700g for 10 min and the supernatant liquid was used as an enzyme source. For determination of ChE activity, 1ml of the enzyme solution and 1ml of 0.004 M acetylcholine bromide were taken in a test tube and incubated at 37°C for 40 min. The residual acetylcholine was determined by the Hestrin method as described in the previous paper[27]. For determination of sensitivity of ChE, an appropriate amount of an inhibitor in acetone was taken in a test tube and 1ml of the enzyme solution was added after the evaporation of acetone. After pre-

incubation at 37°C for 30 min, the substrate was added to determine the residual ChE activity[27].

Results

Effects of synergists on toxicity of malathion and malaoxon

Effects of some synergists to malathion and malaoxon for S, M and P strains are shown in Table 1. Piperonyl butoxide exhibited high antagonistic effect to malathion for all three strains tested. TPP showed synergistic effect to malathion for M and P strains but not for S strain. Any distinct effect of TOCP to malathion was not found for the three strains. EPN-oxon and isopropyl paraoxon markedly synergized the toxicity of malathion, especially isopropyl paraoxon for M and P strains. Any effect of piperonyl butoxide and TPP on the toxicity of malaoxon was not found for the resistant strains.

Penetration and metabolism of malathion

Distribution of radioactivity and mortality in S, M and P strains at various time intervals after topical application of ^{32}P, ^{35}S-labeled malathion are shown in Fig. 1. As radioactivity of ^{32}P decayed during the preparation of radioautograms of TLC plates, Fig. 1 is shown on the basis of ^{35}S. Total recovery includes radioactivities in the external, the toluene and aqueous fractions, and the unextractable residue. Radioactivity remained in plastic case was excluded. Radioactivities of the residues were below 2-3% of the applied dosage and were omitted in Fig. 1.

Disappearance rate of malathion from the surface of insects was faster at the low dosage (0.1μg/insect) than at the high dosage (1.0μg/insect). At 1 hr after application, 30% of the applied dosage remained at the low dosage, whereas 70-80% was recovered at the high dosage. Any difference was not observed in the disappearance rate of malathion among S, M and P strains, regardless of applied dosages. S

Table 1. Effects of synergists on toxicity of malathion and malaoxon for S, M and P strains.

Compound	Manner of application* and ratio of compounds in the mixture	LD_{50} (μg/g)			Cotoxicity coefficient**		
		S	M	P	S	M	P
malathion		0.94	271	52.0			
malaoxon		0.56	4.55	2.93			
EPN-oxon		0.58	13.3	71.4			
isopropyl paraoxon		2.86	27.8	38.7			
malathion	simultaneous application						
+piperonyl	1 : 4	—	—	153			34.0
butoxide	1 : 0.4	—	408	—		66.4	
	pre-application	8.5	466	188	11.1	58.2	27.7
malathion +TOCP	pre-application	0.82	242	58.7	115	112	88.6
malathion +TPP	pre-application	0.83	97.5	18.8	113	278	277
malathion	simultaneous application						
+EPN-oxon	1 : 1	0.68	12.7	18.4	105	200	328
malathion	simultaneous application						
+isopropyl	1 : 1	—	5.75	2.88		876	1540
paraoxon	1 : 4	0.82	—	—	218		
malaoxon	simultaneous application						
+piperonyl putoxide	1 : 10	—	4.00	3.44		114	85.2
malaoxon	simultaneous application						
+TPP	1 : 10	—	4.25	—		107	

* In pre-application, 2 μg of a synergist per female which causes no poisoning, was applied 60 min before the application of an insecticide.

** Cotoxicity coefficients were calculated by the equation of Sun and Johnson[28].

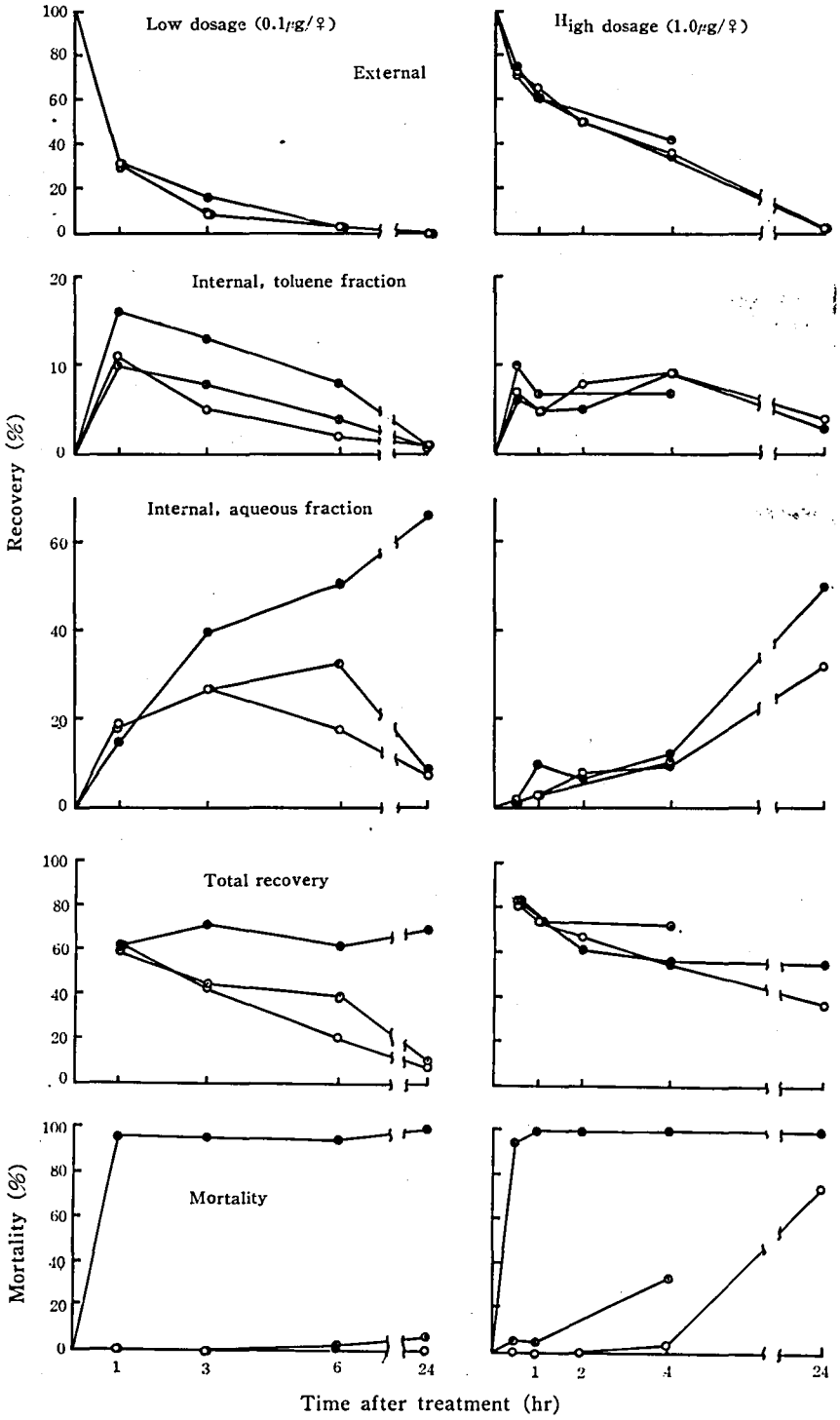


Fig. 1. Penetration and metabolism of malathion, and mortality in S (●), M (○) and P (◉) strains after topical application of radio-labeled malathion.

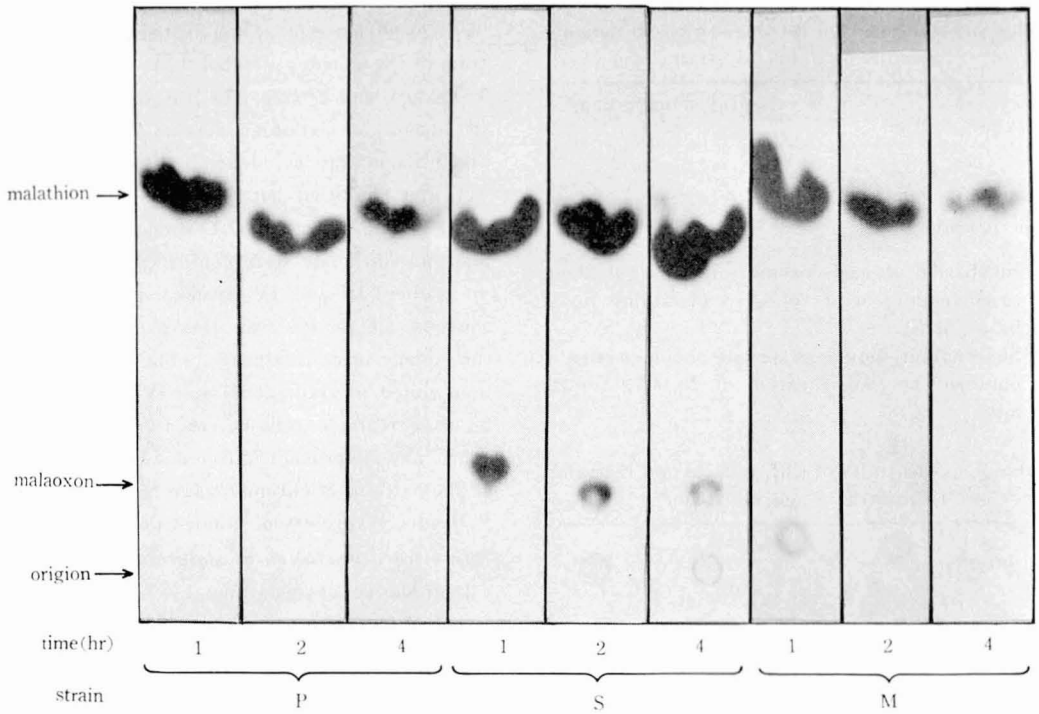


Fig. 2. Radioautograms of TLC of the toluene fraction. The developing solvent system is hexane and acetone (19:1).

Table 2. Amounts of malathion and malaoxon in the toluene fraction at the high dosage (1.0 μg/insect).

Strain	Time elapsed (hr)	Malathion (μg/♀)	Malaoxon* (μg/♀)
S	0.5	0.072131	0.000219
	1	0.055130	0.000335
	2	0.075633	0.000452
	4	0.113165	0.000114
	24	0.034035	0.000173
M	0.5	0.088365	
	1	0.062155	
	2	0.096634	
	4	0.112924	
	24	0.046720	
P	0.5	0.088799	
	1	0.088973	
	4	0.088973	

* Amount of malaoxon could be determined only in S strain, but those for the other strains were impossible, because any spots corresponding to malaoxon were not detected in radioautograms as shown in Fig. 2.

strain was paralyzed or immobilized within 1 hr but any poisoning was not observed in M and P strains in the early stage after treatment. These

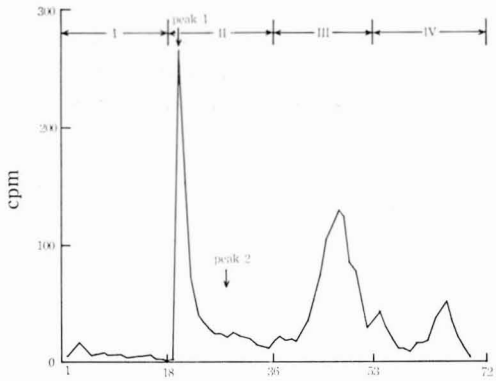


Fig. 3. Ion-exchange chromatogram of the aqueous metabolites on Dowex 1-X8. The sample is the metabolites in S strain 4 hr after topical application of malathion (1.0 μg per female). A series of gradient elution is as following as described by Krueger and O'Brien[26]. Three hundred m*l* of each gradient eluant was used. Gradient I: HCl pH 1.5/HCl pH 1, Gradient II: HCl pH 2 + methanol (1+4)/HCl pH 1+methanol (1+4), Gradient III: HCl pH 1+methanol (1+3)/HCl 1 N +methanol (1+3), Gradient IV: HCl 1 N+acetone (1+3)/HCl conc. +H_2O +acetone (1+1+6).

Table 3. Degradation of malathion by homogenates of S and M strains.

Enzyme solution	Degraded malathion* (μg)	
	S	M
crude homogenate	2.8	5.4
supernatant liquid**	7.0	10.2

* Incubation of 4 m*l* enzyme solution equivalent to 80 females with 20 μg of malathion for 2 hr at 30°C.

** Supernatant liquid of crude homogenates obtained by centrifugation at 20,000g for 20 min.

Table 4. Sensitivity of ChE in S, M and P strains to malathion and malaoxon*.

Inhibitor	I_{50} (M)		
	S	M	P
malathion	2.2×10^{-4}	2.2×10^{-4}	2.2×10^{-4}
malaoxon	8.5×10^{-8}	1.3×10^{-7}	1.0×10^{-7}

* Pre-incubation with an inhibitor for 30 min at 37°C.

observations suggest that penetration of malathion is merely physicochemical phenomenon, and is not affected by poisoning of the leafhopper.

Radioactivity of the toluene fraction in S strain was twice those of M and P strains at the low dosage. Radioactivity of the aqueous fraction increased gradually, except those in M and P strains at the low dosage. The total recovery of radioactivity in M and P strains decreased gradually, though the recovery in S strain was almost constant. These results suggest that metabolites of malathion are steadily excreted in M and P strains but in S strain excretion of metabolites probably decreases or ceases after poisoning, resulting in the accumulation of aqueous metabolites in the body.

Radioautograms of TLC of the toluene fraction at the high dosage are shown in Fig. 2. A clear-cut spot of malathion (Rf value, 0.67) was detected in all three strains tested. Another distinct spot of malaoxon (Rf value, 0.18) was detected only in S strain but not in either M or P strains. Amonuts of malathion and malaoxon calculated on the basis of radioactivities of the TLC plates are shown in Table 2.

A typical pattern of ion-exchange chromatogram of the aqueous metabolites is shown in Fig. 3. Peak 1 and 2 were identified to be monoacid and diacid of malathion, respectively, though in Fig. 3 diacid was not found. Other metabolites were not identified but *O,O*-dimethyl hydrogen phosphorothioate and *O,O*-dimethyl hydrogen phosphorodithioate were confirmed to be eluted in gradient III and IV, respectively. The main aqueous metabolite was the monoacid in the early stage after treatment. The other metabolites eluted in gradient III and IV appeared 1-2 hr after treatment, and increased gradually. However, any significant difference was not found in the patterns of chromatogams among S, M and P strains, regardless of applied dosages.

In vitro degradation of malathion

Degradation of malathion by homogenates of S and M strains is shown in Table 3. The degradation activities by supernatant liquid of homogenates were 2-3 times higher than those by crude homogenates in both strains. The activity in M strain was twice that in S strain, regardless of enzyme sources.

Sensitivity of ChE to malathion and malaoxon

It has been reported in the previous paper[16] that ChE activities of M and P strains are higher than that of S strain. However, sensitivity of ChE to malathion or malaoxon was not different among M, P and S strains as shown in Table 4. Sensitivity of ChE in this leafhopper to malaoxon was approximately 1000 times higher than that to malathion.

Discussion

Although many factors such as cuticular penetration, activation and detoxication of insecticides, and sensitivity of target sites to insecticides are involved in insecticide-resistance, increased activity of degradation is known to be the predominant factor in most of organophosphate-resistance of insects[29].

In this study, any significant difference was not found in either penetration of malathion or sensitivity of ChE to malathion and malaoxon among S, M and P strains. It has been demonstrated by Hama and Iwata[27,30,31] that the altered ChE, which was found in the multi-resistant

strains of the leafhopper, is less sensitive to carbamates, participates mostly in the carbamate-resistance and partially in the resistance to some organophosphates including malathion. However, sensitivities of ChEs of M and P strains to malathion or malaoxon were the same as that of S strain. Therefore, neither penetration of malathion nor alteration of ChE contributes to the malathion-resistance in M and P strains.

Malathion was metabolized relatively quickly both *in vivo* and *in vitro* in all strains tested, though degradation activity of malathion in M and P strains was twice that in S strain. Malathion was metabolized mainly to its monocarboxylic acid in all three strains in the early stage after treatment. It was also shown in this study that TPP and EPN-oxon known as carboxylesterase inhibitors synergized malathion for M and P strains but not for S strain. Therefore, high activity of carboxylesterase is an important factor of the malathion-resistance in M and P strains.

Metabolites eluted in gradient III and IV appeared 1-2 hr after treatment and increased gradually. This result suggests that other enzymes than carboxylesterase also participate in the degradation of malathion. However, a significant difference was not found in the pattern of aqueous metabolites among the three strains.

As shown in Fig. 2 and Table 2, malaoxon was evidently accumulated in the body of S strain but not detected in either M or P resistant strains. It is well known that organophosphates having P=S bond are oxidized to phosphates having P=O bond, potent anticholinesterase agents. In the green rice leafhopper, anticholinesterase activity of malaoxon was shown to be 1000 times higher than that of malathion (Table 4). Therefore, it is evident that intoxication and decease in the leafhopper applied with malathion are directly affected by the amount of malaoxon accumulated at the target site. Consequently, malathion-resistance in M and P strains is attributed to extremely small amount of malaoxon. Such an instance has been demonstrated in the resistance of *Musca domestica* to malathion[1] and diazinon[32,33], and *Culex tarsalis* to malathion[3,5].

From viewpoint of activation and detoxication of malathion, accumulation of malaoxon at the target site may be affected by the following three factors: 1) activation rate of malathion to malaoxon, 2) degradation rate of malaoxon, and 3) degradation rate of malathion.

The above two factors, 1) and 2), relating directly to the accumulation of malaoxon were not elucidated in this study. Since piperonyl butoxide exhibited antagonistic effect to malathion for all strains tested, it is probable that malathion is converted to malaoxon by mixed function oxidases in this leafhopper.

As described in the previous paper[22], both M and P strains are characterized by more than 100 times resistance to many organophosphates having P=S bond but lower resistance to phosphates having P=O bond. In comparison of the resistance patterns of M and P strains to the P=O compounds, M strain is somewhat more resistant to malaoxon, vamidothion and Fujithion [*S*-(*p*-chlorophenyl) *O*, *O*-dimethyl phosphorothiolate] than P strain, but conversely the level of resistance to methyl paraoxon, paraoxon, fenitrothion-oxon and EPN-oxon is extremely higher in P strain than in M strain[22]. From these results, it is possible that M and P strains may degrade the P=O compounds, if so, more than one enzyme will be involved in the degradation. Degradation of malaoxon undoubtedly affects the malathion-resistance. It has been reported that malaoxon is degraded in the resistant insects[1,3,5,10,34,35], but not in the green rice leafhopper[12], Main and Dauterman[36] showed that carboxylesterase from rat liver hydrolyzed malaoxon, and was simultaneously irreversibly inhibited by phosphorylation. Considering malaoxon the actual toxicant of malathion, a small degradation of malaoxon may be important, as well as a large degradation of malathion. Then degradation of malaoxon in this leafhopper needs to be investigated details.

Finally, degradation rate of malathion affects indirectly accumulation of malaoxon, i. e., high degradation rate of malathion results in decrease of the substrate malathion on the reaction of activation. This factor has been already discussed above.

On the whole, the degradation activity of malathion in M and P strains was at most twice that

in S strain both *in vivo* and *in vitro*, and synergistic effects of TOCP, TPP and EPN-oxon to malathion were not clear-cut as compared with those in the other malathion-resistant insects. Besides, it was shown[22,37] that in this leafhopper selection with malathion resulted in inducing a high levels of resistance to many other organophosphate. In these regards, extremely small amount of malaoxon in the resistant leafhopper seems not to be explained solely by the increased activity of carboxylesterase. It is probable that the hydrolysis of malathion by carboxylesterase and other factor(s) such as degradation of malaoxon may be responsible for small amount of malaoxon or high level of malathion-resistance in the leafhopper.

Acknowledgement: The authors wish to thank Drs. T. Shishido and J. Fukami for kindly supplying chemicals.

Summary

In order to clarify mechanism of the malathion-resistance in the green rice leafhopper, effects of some synergists, penetration and metabolism of malathion, and sensitivity of ChE were determined using two organophosphate-resistant strains (M and P) and one susceptible strain (S).

Any distinct difference was not found in penetration of malathion and sensitivity of ChE to malathion and malaoxon among S, M and P strains.

Although malathion was metabolized relatively quickly to aqueous metabolites in the three strains both *in vivo* and *in vitro*, the degradation activity in M and P strains was twice that in S strain. The main aqueous metabolite was malathion monoacid. Other unknown metabolites appeared 1-2 hr after treatment and increased gradually, but a distinct difference was not found in the patterns of aqueous metabolites among S, M and P strains.

Malaoxon was evidently accumulated in the body of S strain but not detected in M and P strains. Consequently, extremely small amount of malaoxon in the resistant strains was explainable to the resistance to malathion in these resistant leafhoppers.

References

1) Matsumura, F. and C. J. Hogendijk: *Ent. exp. appl.*, 7, 179 (1964).
2) Welling, W. and P. T. Blaakmeer: In '*Insecticide Resistance, Synergism, Enzyme Induction*' (A. S. Tahori, ed.), 61-75, Gordon and Breach, London (1971).
3) Matsumura, F. and A. W. A. Brown: *J. Econ. Entomol.*, 54, 1176 (1961).
4) Matsumura, F. and A. W. A. Brown: *J. Econ. Entomol.*, 56, 381 (1963).
5) Bigley, W. S. and F. W. Plapp: *J. Insect Physiol.*, 8, 545 (1962).
6) Townsend, M. G. and J. R. Busvine: *Ent. exp. appl.*, 12, 243 (1969).
7) Dyte, C. E. and D. G. Rowlands: *J. Stored Prod. Res.*, 4, 157 (1963).
8) Busvine, J. R. and M. Feroz: In '*Insecticide Resistance, Synergism, Enzyme Induction*' (A. S. Tahori, ed.), 1-28, Gordon and Breach, London (1971).
9) March, R. B.: *Misc. Publ. Entomol. Soc. Amer.*, 1, 13 (1959).
10) Heuvel, M. J. van den and D. G. Cochran: *J. Econ. Entomol.*, 58, 872 (1965).
11) Kojima, K., T. Ishizuka and S. Kitakata: *Botyu-Kagaku*, 28, 17 (1963).
12) O'Brien, R. D.: '*Insecticides, action and metabolism*' 332pp Academic Press, New York (1967).
13) Hayashi, M. and M. Hayakawa: *Jap. J. appl. Ent. Zool.*, 6, 250 (1962).
14) Kasai, T. and Z. Ogita: *SABCO J.*, 1, 130 (1965).
15) Ozaki, K. and H. Koike: *Jap. J. appl. Ent. Zool.*, 9, 53 (1965).
16) Hama, H. and T. Iwata: *Appl. Ent. Zool.*, 7, 177 (1972).
17) Ozaki, K., Y. Kurosu and H. Koike: *SABCO J.*, 2, 98 (1966).
18) Ohkawa, H., M. Eto, Y. Oshima, F. Tanaka and K. Umeda: *Botyu-Kagaku*, 33, 139 (1968).
19) Miyata, T. and T. Saito: *J. Pesticide Sci.*, 1, 23(1976).
20) Ozaki, K. and Y. Kurosu: *Jap. J. appl. Ent. Zool.*, 11, 145 (1967).
21) Iwata, T. and H. Hama: *Botyu-Kagaku*, 36,

174 (1971).
22) Iwata, T. and H. Hama: *Botyu-Kagaku*, 42, 181.
23) Casida, J. E.: *Acta. Chem. Scand.*, 12, 1691 (1958).
24) March, R. B., T. R. Fukuto, R. L. Metcalf and M. G. Maxon: *J. Econ. Entomol.*, 49, 185 (1956).
25) Foss, O.: *Acta Chem. Scand.*, 1, 8 (1947).
26) Krueger, H. R. and R. D. O'Brien: *J. Econ. Entomol.*, 52, 1063 (1959).
27) Hama, H. and T. Iwata: *Appl. Ent. Zool.*, 6, 183 (1971).
28) Sun, Y. P. and F. R. Johnson: *J. Econ. Entomol.*, 53, 887 (1960).
29) Perry, A. S. and M. Agosin: In '*The Physiology of Insecta*' (M. Rockstein, ed.), Vol. 6, 3-121, Academic Press, New York (1974).
30) Iwata, T. and H. Hama: *J. Econ. Entomol.*, 65, 613 (1972).
31) Hama, H. and T. Iwata: In preparation.
32) Krueger, H. R., R. D. O'Brien and W. C. Dauterman: *J. Econ. Entomol.*, 53, 25 (1960).
33) Shono, T.: *Jap. J. Sanit. Zool.*, 24, 249 (1974).
34) Oppenoorth, F. J. and K. van Asperen: *Ent. exp. appl.*, 4, 311 (1961).
35) Welling, W., A. W. de Vries and S. Voerman: *Pestic. Biochem. Physiol.*, 4, 31 (1974).
36) Main, A. R. and W. C. Dauterman: *Can. J. Biochem.*, 45, 757 (1967).
37) Tanaka, F., S. Kitakata and K. Umeda: *Annual Meeting of Jap. Soc. Appl. Ent. Zool.* (1967).

抄　　録

マメコガネ雌成虫の性フェロモンの構造 対掌体による雄の誘引阻害

Identification of the Female Japanese Beetle Sex Pheromone: Inhibition of Male Response by an Enantiomer. J. H. Tumlinson, M. G. Klein, R. E. Doolittle, T. L. Ladd and A. T. Proveaux, *Science*, 197, 789 (1977).

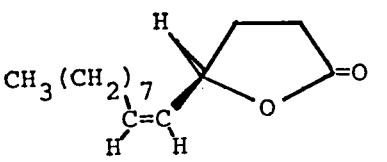
マメコガネ Japanese beetles (*Popillia japonica*) の処女雌より性フェロモンを単離しその化学構造を決定した.

性フェロモンのサンプリングは 3,000 匹ほどの処女雌を大型の PC 用ガラス容器内で飼育し，毎朝虫を他の同じ容器に移し，飼育をおえた容器を 30m*l* のベンゼンで 3 回洗う，さらに毎夕同様の操作をおこない新しい容器にはエサとしてリンゴの薄切を入れておく．その洗液を集め粗フェロモンを得その濃度の表示法は female-day equivalent (FD) とした.

野外試験は 50～100FD に相当するベンゼン洗液をガラスのペトリ皿にとり，溶媒をとばした後それをゴルフコースのフェアウエーなどに設置する．それに対して 5 分以内に誘引された雄成虫を数え，その数を同区域内でカゴに入れた 3 匹の処女雌に対し同時間内に誘引された雄成虫の数と比較する．という方法でおこない単離の各段階の活性はすべてこの方法でチェックした.

上記の方法で集めた粗フェロモンを LC および GC により精製し性フェロモンを単離し，MS, IR, NMR などの機器分析その他により (Z)or (E)-5-(1-Decenyl) dihydro-2 (3H)-furanone と決定した．両化合物は分子中に不斉炭素原子を持ちそれぞれ 2 つの対掌体が存在するが，まずそれらをそれぞれラセミ混合物として合成し，その GC 上の挙動を 5 種のカラムを使って天然物と比較したところ Z 体の Rt が完全に一致した．しかし，Z 体のラセミ混合物を使っての野外試験は雄成虫を誘引できなかった．そこでさらに Z，E 異性体および飽和体と，それぞれのエナンチオマー計 6 種を立体特異的に合成した．R(−) Gulutamic aicd より合成した (R, Z)-5-(1-Decenyl) dihydro-2 (3H)-furanone の純品は処女雌および単離したフェロモンと同等の誘引力があった．しかるにこの（R, Z）体の誘引力は 1％程度の（S, Z）体の存在によりあきらかに阻害され，その度合は（S, Z）体の添加量の増加とともに大きくなった．また E 体および飽和体の性フェロモン類縁化合物は処女雌抽出物中に存在するが，それらの物質の作用ははっきりしない.

（山岡亮平）



(R,Z)-5-(1-Decenyl)dihydro-2(3H)-furanone