Studies on the Inheritance of Carbamate-resistance in the
Green Rice Leafhopper, *Nephotettix cincticeps* UHLER
(Hemiptera : Cicadellidae)
Relationships between Insensitivity of Acetylcholinesterase
and Cross-resistance to Carbamate and Organophosphate
Insecticides¹

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Susceptibility to three carbamate insecticides and sensitivity of acetylcholinesterase (AChE) to a carbamate in susceptible and resistant strains and their F₁, F₂ and backcross progenies of the green rice leafhopper were determined. The results suggested that the carbamate-resistance is mainly controlled by an incompletely dominant autosomal factor, and that the gene responsible for the insensitivity of AChE is probably on the same autosome. Evidence based on the results of repeated backcrosses to the susceptible strain under selection with a carbamate suggested that both the carbamate-resistance and the carbamate-insensitivity of AChE are due to the same gene. It was also considered that non-specific esterases do not play a role in carbamate-resistance.

The relationships between the insensitivity of AChE and cross-resistance in the multi-resistant leafhopper was analyzed using a special strain in which the gene for the insensitivity of AChE was introduced by repeated backcrosses into the background of the susceptible strain. By comparing susceptibility to insecticides and sensitivity of AChE to inhibitors in the substrain versus the two parental strains, it was found that resistance to the carbamates propoxur, Tsumacide® (3-methylphenyl *N*-methylcarbamate), carbaryl, Bassa® (2-*sec*-butylphenyl *N*-methylcarbamate) and Knockbar® (3-*tert*-butylphenyl *N*-methylcarbamate) is caused mostly by the insensitivity of AChE. In contrast, resistance to carbanolate, Hydrol® (4-diallylamino-3,5-dimethylphenyl *N*-methylcarbamate) and methomyl was influenced little or not at all by the alteration of AChE. The altered AChE was also found to contribute a part of the resistance to organophosphates such as malathion, phenthoate, fenitrothion and Fujithion® (*S*-(*p*-chlorophenyl) *O,O*-dimethyl phosphorothiolate), but had no influence on the resistance to pyridafenthion, propaphos, acephate and diazinon. Diazoxon, pyridafenthion-oxon and propaphos inhibited the altered AChE of the resistant strain more strongly than the normal enzyme of the susceptible strain.

INTRODUCTION

In the green rice leafhopper, *Nephotettix cincticeps* UHLER, malathion-resistance occurred in 1961 and was followed by widespread resistance toward other organophos-

¹ The summary of this work was presented at the Annual Meeting of Japan Soc. Appl. Ent. Zool., Sapporo, August 29, 1974. The work is published new after further experimentation.

phates. Since carbamate insecticides had been used extensively and frequently as substitutes for organophosphates, resistance to this group also occurred after 4 or 5 years. Consequently, most carbamate-resistant populations are also resistant to organophosphate insecticides (IWATA and HAMA, 1971, 1972).

Malathion-resistance in this leafhopper is due mainly to increased activity of carboxylesterase hydrolyzing malathion to non-toxic acids (KOJIMA et al., 1963; MIYATA and SAITO, 1976; HAMA et al., 1977). Though the mechanism of resistance toward the other organophosphates has not been elucidated, it appears to involve increased activity of detoxication enzymes. On the other hand, the carbamate-resistance is due to mainly the insensitivity of acetylcholinesterase (AChE) to inhibition by carbamates. HAMA and IWATA (1971) showed that AChE of a multi-resistant Nakagawara(N) strain is less sensitive to carbamates as compared with that of a susceptible strain. This finding was substantiated by additional similar observations (HAMA and IWATA, 1973). Several populations collected in the districts of Shikoku, Kyushu and southern Honshu showed the resistance spectra to carbamates to be qualitatively and quantitatively similar to those of the N strain. The AChEs of these populations were insensitive to carbamates to the same extent to that of the N strain. Furthermore, populations which developed moderate resistance to carbamates were found to consist of at least two phenotypes, i.e. relatively carbamate-susceptible and -resistant as judged by their dosage-mortality relationships (HAMA and IWATA, 1973). These observations suggest that inheritance of the carbamate-resistance in this leafhopper is relatively simple.

An alteration of AChE is also presumed to contribute in the resistance to some organophosphates. For example, AChE of the N strain is 6 and 18 times less sensitive to malathion and malaoxon, respectively, than that of a susceptible strain (IWATA and HAMA, 1972). Furthermore, the levels of resistance to organophosphates had increased after the occurrence of carbamate-resistance (IWATA and HAMA, 1971).

The present paper describes the mode of inheritance of carbamate-resistance and its relation to the insensitivity of AChE, and the relationships between insensitivity of AChE and cross-resistance in the multi-resistant green rice leafhopper. Results concerning propaphos have been reported earlier (HAMA, 1975).

MATERIALS AND METHODS

Strains. Miyagi susceptible (S) and Nakagawara multi-resistant (N) strains of the green rice leafhopper, *Nephotettix cincticeps* UHLER, were used as the parental strains (HAMA and IWATA, 1971). Both strains were collected in the field and have been reared at $27 \pm 1.5^\circ\text{C}$. The N strain has been selected in several generations with a carbamate by the manner described below. This strain shows high resistance to carbamate as well as organophosphate insecticides and appears to be nearly homogeneous in susceptibility to carbamates (IWATA and HAMA, 1972; HAMA and IWATA, 1973). A substrain Rmc was produced from S and N strains according to a technique of repeated backcrossing to S strain under selection with a carbamate (HAMA, 1975) as shown in Fig. 1. After the process of backcrossing followed by selection with Bassa was repeated for four successive generations, the surviving heterozygotes of the 4th backcross progeny were mass-inbred. The resulting progeny was selected with the discriminating dosage of Bassa over two generations in order to obtain homozygous

Fig. 1. Process of producing a substrain, Rmc, from S and N strains, by repeated back-crossing to S strain under selection with a carbamate, Bassa.

resistant individuals. This strain has been selected with Bassa in several generations. Adults of 3 to 8 days old were used in all experiments.

Crossing procedure. Mass-mating was adopted for all crossings. Virgin females were obtained by the following procedure: mature nymphs were collected into a wire net-cage (35 cm × 40 cm × 30 cm), in which rice seedlings were placed. Emerging females were transferred to another cage every day and at the same time emerging males were removed. The females were maintained several days for ageing. One hundred to 200 virgin females thus obtained from one strain (or progeny) were mass-crossed with 50 to a 100 males from another.

Selection with a carbamate insecticide. In order to remove susceptible and heterozygous phenotypes in a progeny, selection with Bassa was conducted. Bassa was used as a selecting agent, because the N strain shows more than 100-fold resistance to Bassa (HAMA and IWATA, 1971). Mature nymphs of mixed sexes were collected into the cage containing the rice seedlings, and 30–40 ml of diluted emulsion of Bassa was sprayed directly to the leafhoppers and the seedlings. The cage was kept at $27 \pm 1.5^\circ\text{C}$ and survivors were transferred to another cage 1 day after treatment. From preliminary tests, it was determined that the discriminating dosages of Bassa that kill only susceptible phenotypes, or both susceptible and heterozygous phenotypes were 50 ppm and 70 ppm, respectively.

Insecticides. The following 8 carbamate and 11 organophosphate insecticides were used in bioassay tests. Carbamates: propoxur (pure), Bassa® (2-*sec*-butylphenyl *N*-methylcarbamate, 97%), carbaryl (98%), carbanolate (2-chloro-4,5-dimethylphenyl *N*-methyl carbamate, pure), Hydrol® (4-diallylamino-3,5-dimethylphenyl *N*-methylcarbamate pure), Tsumacide® (3-methylphenyl *N*-methylcarbamate, 97%), Knockbar® (3-*tert*-butylphenyl *N*-methylcarbamate, pure), methomyl (90.1%). Organophosphates:

malathion(96.3%), phenthoate(pure), dimethoate(98%), mecarbam(diethyl *S*-(*N*-ethoxycarbonyl-*N*-methylcarbamoylmethyl) phosphorothiolothionate, 81%), fenitrothion(95.6%). Fujithion®(*S*-(*p*-chlorophenyl)*O,O*-dimethyl phosphorothiolate, 85%), chlorfenvinphos(95%), diazinon(95.3%), pyridafenthion(diethyl 3-oxo-2-phenyl-2*H*-pyridazin-6-yl phosphorothionate, 99.0%), propaphos(di-*n*-propyl *p*-methylthiophenyl phosphate, 94.4%), acephate(*O,S*-dimethyl *N*-acetylphosphoramidothiolate, pure).

Inhibitors. Recrystallized propoxur and following 7 organophosphates having a P=O bond were used as inhibitors for determination of sensitivity of AChE: malaoxon, fenitrothion-oxon, chlorfenvinphos, diazoxon, pyridafenthion-oxon, propaphos, acephate. These compounds were purified by thin-layer chromatography on silica gel plates prior to use.

Bioassay tests. The topical application technique was adopted as described previously(HAMA and IWATA, 1971). A drop of acetone solution of an insecticide(0.5 μ l for female or 0.2 μ l for male) was applied on the dorsal surface of the adult under CO₂ anesthesia. The treated insects were placed in a plastic case containing the rice seedlings, and kept at 27 $\pm$ 1.5°C. Mortality was counted 24 hr after treatment. At each concentration of an insecticide 15 to 20 adults were used, and tests were repeated at least three times. Dosage-mortality regression lines and LD₅₀ values for three strains and F₁ progeny were calculated by probit analysis. Females were used in all tests unless otherwise stated.

Determination of AChE and AliE activities. One hundred females were homogenized in 10 ml of 0.067 M phosphate buffer, pH 7.2, in a glass homogenizer. The homogenate was filtered through a nylon cloth and the filtrate was used as the enzyme source. AChE activity and non-specific esterase(AliE) activity were determined by the HESTRIN method (HESTRIN, 1949) by using acetylcholine bromide and methyl-*n*-butyrate as substrates, respectively(HAMA and IWATA, 1971, 1972).

Determination of sensitivity of AChE to inhibitors. The pre-inhibition technique was adopted for all tests(HAMA and IWATA, 1971). The enzyme solution used in the determination of sensitivity of AChE to propoxur was the same as mentioned above. The appropriate amount of propoxur in acetone was taken into a test tube, and the enzyme solution was added after evaporation of acetone, and incubated for 15 or 30 min at 37°C. The substrate was then added, and incubated further for 30 or 40 min. The residual AChE activity was determined by the HESTRIN method.

For determination of sensitivity of AChE to organophosphates, the enzyme source was prepared by the following procedure and AChE activity was measured by the ELLMAN method (ELLMAN et al., 1961) as previously described (HAMA, 1975). Adults of mixed sexes were homogenized in 10-fold distilled water and then centrifuged at 700 *g* for 10 min. The supernatant liquid was further centrifuged at 105,000 *g* for 60 min. The precipitate, which shows a high AChE activity and little AliE activity (HAMA, 1975, 1976), was suspended in a small volume of distilled water, and used as the AChE source. The appropriate amount of an organophosphate in acetone was taken in a test tube, in which 4.8 ml of 0.1 M phosphate buffer(pH 7.4) was added after evaporation of acetone, and then 0.2 ml of the enzyme solution was added. After incubation for 15 min at 30°C, 0.1 ml of 0.03 M acetylthiocholine iodide and 0.1 ml of 0.02 M 5,5-dithio-bis-2-nitrobenzoic acid(DTNB) were added, and incubated further for 10 min to determine the residual AChE activity. The reaction was stopped by adding 0.1 ml of 0.01 M eserine sulfate and the absorbance of the solution was measured

at 412 nm.

RESULTS

Susceptibility of parental strains and their progenies to carbamate insecticides

Susceptibility of S and N strains, and their F_1 , F_2 and backcross progenies to propoxur, Tsumacide and carbaryl is shown in Table 1 and Figs 2, 3 and 4. By comparison with the S strain, females of the N strain showed 122, 17 and 67-fold resistance to propoxur, Tsumacide and carbaryl, respectively; and males showed 204, 14 and 53-fold resistance.

The dosage-mortality lines of F_1 progeny with the three carbamates were almost linear, and nearer those of N strain rather than the S strain. The F_1 progeny from the cross $N\text{♀} \times S\text{♂}$ seemed a little more resistant to propoxur than the progeny from $S\text{♀} \times N\text{♂}$, but the differences were not statistically significant at 5% level of probability. Therefore, the dosage-mortality curve of F_1 calculated from the pooled mortalities is presented in Fig. 2. According to the method of STONE(1968), the degrees of dominance in the resistance to propoxur, Tsumacide and carbaryl were calculated to be 0.44, 0.35 and 0.31, respectively, indicating incomplete dominance.

If carbamate-resistance in the N strain is controlled by a single genetic factor on an autosome, backcrossing of F_1 to the S strain should yield a progeny consisting of susceptible and heterozygous individuals in the ratio of 1:1 according to simple Mendelian inheritance. Likewise, the F_2 progeny should consist of susceptible, heterozygous and resistant individuals in the ratio of 1:2:1. Assuming monofactoriality, the

Table 1. SUSCEPTIBILITY OF S AND N STRAINS OF *Nephotettix cincticeps*, AND THEIR F_1 PROGENY TO PROPOXUR, TSUMACIDE AND CARBARYL

Insecticide	Strain or progeny	Sex	Dorsage-mortality regression formula ^a	LD ₅₀ ($\mu\text{g}/\text{adult}$)	Ratio of LD ₅₀ (strain or progeny/S strain)
Propoxur	S	♀	$Y=5.90X-0.73$	0.0094	
		♂	$Y=2.39X+4.06$	0.0025	
	N	♀	$Y=3.76X-6.51$	1.15	122
		♂	$Y=3.04X-3.24$	0.51	204
	$F_1(S\text{♀} \times N\text{♂})$	♀	$Y=2.15X-0.06$	0.23	24
	$F_1(N\text{♀} \times S\text{♂})$	♀	$Y=2.37X-0.98$	0.33	35
	F_1^b	♀	$Y=2.10X-0.18$	0.30	32
Tsumacide	S	♀	$Y=3.43X+0.74$	0.018	
		♂	$Y=2.50X+3.24$	0.0051	
	N	♀	$Y=2.93X-2.27$	0.30	17
		♂	$Y=3.53X-1.48$	0.069	14
	$F_1(S\text{♀} \times N\text{♂})$	♀	$Y=2.67X-0.55$	0.12	6.7
Carbaryl	S	♀	$Y=3.28X+3.57$	0.0027	
		♂	$Y=3.45X+1.53$	0.0010	
	N	♀	$Y=4.66X-5.45$	0.18	67
		♂	$Y=2.74X+0.29$	0.053	53
	$F_1(S\text{♀} \times N\text{♂})$	♀	$Y=2.68X+0.65$	0.042	16

^a $Y=\text{probit}$, $X=\log \text{dosage} \times 10^3$.

^b Calculated from the pooled mortalities of the reciprocal crosses of S and N strains.

Fig. 2. Dosage-mortality relationships of progenies derived from the cross S and N strains of *Nephrotettix cincticeps* tested with propoxur. The dosage-mortality line of F_1 was calculated from the pooled mortalities of $S \varphi \times N \delta$ ($\bullet$) and $N \varphi \times S \delta$ ($\circ$). Pooling was done because regression formulas of F_1 from the reciprocal crosses did not differ significantly from each other at 5% level of probability. Expected mortality curves of B_1 and F_2 (---) were calculated from the regression formulas of S, N and F_1 on the basis of monofactorial inheritance. Observed mortalities of B_1 , $S \varphi \times F_1$ ($S \varphi \times N \delta$) δ ($\circ$) and $S \varphi \times F_1$ ($N \varphi \times S \delta$) δ ($\odot$), and F_2 ($\circ$) derived from $S \varphi \times N \delta$. $\uparrow$, 100% mortality; $\downarrow$, 0% mortality. Significant deviations at 5% level of probability of the observed from the expected are circled.

Fig. 3. Dosage-mortality relationships of progenies of *Nephrotettix cincticeps* derived from the cross $S \varphi \times N \delta$ tested with Tsumacide. Expected mortality curves of B_1 and F_2 (---) were calculated from the regression formulas of S, N and F_1 on the basis of monofactorial inheritance. Observed mortalities of B_1 ($S \varphi \times F_1 \delta$, $\circ$) and F_2 ($\circ$). $\uparrow$, 100% mortality; $\downarrow$, 0% mortality. The deviation of the observed from the expected mortalities was not significant at all the doses.

Fig. 4. Dosage-mortality relationships of progenies of *Nephrotettix cincticeps* derived from the cross $S \text{♀} \times N \text{♂}$ tested with carbaryl. Expected mortality curve of B_1 and F_2 (-----) were calculated from the regression formulas of S , N and F_1 on the basis of monofactorial inheritance. Observed mortalities of B_1 ($S \text{♀} \times F_1 \text{♂}$, $\bigcirc$) and F_2 ($\bigcirc$). $\uparrow$, 100% mortality; $\downarrow$, 0% mortality. The deviation of the observed from the expected mortalities was not significant at all the doses.

expected percent mortalities in the backcross (B_1) and F_2 progenies were calculated from the regression lines of the two parental strains and their F_1 hybrid by the method of Hoskins (Tsukamoto, 1963), and are shown as dotted lines in Figs 2, 3 and 4. The observed mortalities of B_1 and F_2 progenies agreed with the expected mortalities at most of the doses except as shown by circles ($\uparrow \downarrow$). These results suggest that the carbamate-resistance of N strain is controlled mainly by an incompletely dominant, autosomal single allele.

Sensitivity of AChE in parental strains and their progenies to propoxur

Sensitivity of AChE in S and N strains and their progenies to propoxur is shown

Fig. 5. Sensitivity of AChE in F_1 ($S \text{♀} \times N \text{♂}$, $\blacktriangle$), B_1 ($S \text{♀} \times F_1 \text{♂}$, $\bigcirc$), F_2 ($\triangle$) derived from $S \text{♀} \times N \text{♂}$, and Rmc strain ($\bigcirc$) of *Nephrotettix cincticeps* to propoxur. Incubation with propoxur for 30 min at 37°C .

in Fig. 5. Relationships between concentration of propoxur (in log scale) and inhibition of AChE activity were almost linear in all the progenies in given concentrations. The AChE I_{50} values of propoxur were 1.3×10^{-5} M and 1.5×10^{-3} M for the S and N strains, respectively. AChEs of F_1 and F_2 were much lower in sensitivity than that of the S strain. Sensitivity of AChE in B_1 was intermediate between those of S and F_1 .

Relationship between the carbamate-resistance and the insensitivity of AChE

CROW (1957) and OPPENOORTH (1965) recommended repeated backcrossing to a susceptible strain under selection to confirm monofactorial inheritance of insecticide-resistance. The authors adopted this technique to confirm this assumption. As shown in Fig. 1, males of F_1 ($S \text{♀} \times N \text{♂}$) were backcrossed to females of the S strain. The backcross progeny (B_1) was selected as already described with the discriminating

Fig. 6. Dosage-mortality relationships of backcross progenies of *Nephrotettix cincticeps* on propoxur. Expected mortality curve of B_1 (-----) was calculated on the basis of monofactorial inheritance. Observed mortalities of B_2 (●), B_3 (○) and B_4 (△).

Table 2. AChE ACTIVITY AND ITS SENSITIVITY TO PROPOXUR, AND AliE ACTIVITY IN BACKCROSS PROGENIES AND Rmc STRAIN OF *Nephrotettix cincticeps* IN COMPARISON WITH THOSE IN S AND N STRAINS

Backcross progeny or strain	AChE activity (μ M acetylcholine/ 10 ♂ /30 min at 37°C)	Inhibition of AChE by 10^{-4} M propoxur for 15 min (%)	AliE activity (μ M methyl- <i>n</i> -butyrate/ 10 ♂ /30 min at 37°C)
B_1	2.31 ^a	52.0 ^a	3.71 ^a
B_2	2.26	44.8	3.16
B_3	2.21	43.0	1.37
B_4	2.21	51.0	1.93
Rmc	— (3.19) ^b	11.5	1.13
S	2.09(2.94)	76.9	0.70
N	3.02(3.30)	27.6	15.59

^a Mean values on 4 replicates.

^b Values in parentheses are μ M acetylcholine/10 ♀ /40 min at 37°C.

dosage of Bassa sufficient to kill susceptible nymphs, and the surviving males (assumed to be heterozygotes) were again backcrossed to the S strain. Such a backcrossing to S strain followed by selection with Bassa was repeated throughout four successive generations.

If the carbamate-resistance is monofactorial and due to the insensitivity of AChE, each backcross progeny must segregate into two phenotypes and its AChE must be intermediate in sensitivity between those of S and N strains. As shown in Fig. 6 and Table 2, each backcross progeny segregated into two phenotypes by a distinct inflection in the region of 50% of the dosage-mortality line. Likewise, sensitivity of AChE to propoxur in each backcross progeny was intermediate between those of the parental populations (Table 2). Although there is no information on crossing-over in this leafhopper, it is considered that the carbamate-resistance is mainly controlled by an incompletely dominant single allele on one of the autosomes, and that the gene responsible for the insensitivity of AChE is probably the same.

AChE and AliE activities in Rmc strain

AChE and AliE activities in the Rmc substrain are shown in Table 2. AChE activity was intermediate between those of S and N, and its sensitivity to propoxur was extremely low as compared to that of the S strain (Table 2 and Fig. 5). The I_{50} value of propoxur for AChE in Rmc was 3.0×10^{-3} M as compared to 1.5×10^{-3} M in strain N.

The AliE activity in the N strain was very high as previously reported (HAMA and IWATA, 1972), but extremely low in the Rmc strain.

Susceptibility of Rmc strain to carbamate insecticides

The dosage-mortality relationships of Rmc strain for carbamate insecticides were almost linear. The LD_{50} values obtained with carbamates on strains Rmc, S and N are presented in Table 3. The resistance levels of Rmc strain toward propoxur, Tsumacide, carbaryl, Bassa and Knockbar were nearly comparable to those of the parental N strain, whereas toward carbanolate, Hydrol and methomyl they were much lower than in the N strain.

Table 3. SUSCEPTIBILITY OF Rmc STRAIN OF *Nephotettix cincticeps* TO CARBAMATE INSECTICIDES IN COMPARISON WITH THOSE OF S AND N STRAINS

Insecticide	LD_{50} ($\mu\text{g}/\varphi$)			Ratio of LD_{50}	
	S ^a	Rmc	N	Rmc/S	N/S
Propoxur	0.0094	1.40	1.15	149	122
Tsumacide	0.018	0.25	0.30	14	17
Carbaryl	0.0027	0.13	0.18	48	67
Bassa	0.0072	0.65	0.75	90	104
Carbanolate	0.0028	0.044	0.17	16	61
Hydrol	0.0028	0.011	0.092	3.9	33
Knockbar	0.0070	0.33	0.38	47	54
Methomyl	0.0013	0.0023	0.015	1.8	12

^a Values for S strain were recalculated from the data of IWATA and HAMA (1971), except that of Knockbar.

Susceptibility of Rmc strain to organophosphate insecticides

The dosage-mortality relationships of Rmc strain for some organophosphate insecticides were not linear (Fig. 7). Susceptibility of Rmc strain to all organophosphates tested was considerably higher than that of N strain. The Rmc strain was almost as susceptible to pyridafenthion, propaphos and acephate as the S strain.

Sensitivity of AChE in Rmc strain to organophosphates

The sensitivity of AChE of strains Rmc and S to organophosphates having P=O

Fig. 7. Dosage-mortality relationships of Rmc strain of *Nephrotettix cincticeps* on organophosphate insecticides in comparison with those of S and N strains. ⬆, 100% mortality; ⬇, 0% mortality.

Table 4. SENSITIVITY OF AChE IN S AND Rmc STRAINS OF *Nephotettix cincticeps* TO ORGANOPHOSPHATES

Inhibitor	I ₅₀ (M) ^a		Ratio of I ₅₀ Rmc/S
	S	Rmc	
Malaoxon	2.5×10^{-8}	3.0×10^{-7}	12
Fenitrothion-oxon	2.5×10^{-7}	2.0×10^{-6}	8
Chlorfenvinphos	3.2×10^{-7}	1.1×10^{-6}	3.4
Diazoxon	1.5×10^{-7}	3.0×10^{-8}	0.2
Pyridafenthion-oxon	1.2×10^{-7}	3.6×10^{-8}	0.3
Propaphos	$>2 \times 10^{-4}$	7.5×10^{-5}	<0.4
Acephate	$>2 \times 10^{-4}$	$>2 \times 10^{-4}$	—

^a Concentration of inhibitor giving 50% inhibition of AChE activity. Incubation with an inhibitor for 15 min at 30°C.

bond is shown in Table 4. The enzyme of Rmc was about 10 times less sensitive to malaoxon and fenitrothion-oxon than that of the S strain, but conversely it was 5, 3.3 and >2.7 times more sensitive to diazoxon, pyridafenthion-oxon and propaphos, respectively.

Anti-acetylcholinesterase activity of propaphos and acephate was rather poor as compared with the other organophosphates. Inhibition rate of AChE by 2×10^{-4} M propaphos was 10% and 70% for S and Rmc strains respectively, and inhibition by 2×10^{-4} M acephate was not detected for the both strains.

DISCUSSION

Analysis of dosage-mortality relationships of F₁, F₂ and backcross progenies derived from the cross of S and N strains, has revealed that resistance of the N strain to carbamates such as propoxur, Tsumacide and carbaryl, is controlled mainly by an incompletely dominant, autosomal single allele, and that the gene responsible for the insensitivity of AChE is probably the same.

OZAKI et al. (1966) demonstrated that a high AliE activity is closely linked with the malathion-resistance in this leafhopper. N strain shows a resistance to both carbamates and organophosphates including malathion, and has a considerably high AliE activity (HAMA and IWATA, 1972). However, AliE activity of the backcross progenies has decreased with repeated backcrossing to the S strain, whereas propoxur resistance has persisted (Table 2 and Fig. 6). The activity of strain Rmc was extremely low as compared with that of strain N (Table 2). These findings indicate that AliE is not involved in carbamate-resistance in this leafhopper.

In order to analyze to what extent the insensitivity of AChE contributes to multiple resistance of strain N, the laboratory-selected strain Rmc was used. This sub-strain showed a resistance to propoxur and Bassa of the same degree as found in strain N (Table 3) and its AChE was less sensitive to propoxur than was the case in strain N (Fig. 5). However, the AliE activity of strain Rmc was considerably lower than that of strain N (Table 2). Therefore, it might be possible to introduce the gene responsible for insensitivity of AChE into the genome of strain S.

The Rmc strain showed high resistance to propoxur, Tsumacide, carbaryl, Bassa

and Knockbar to a level almost equal to that of the N strain, whereas the resistance levels to carbanolate, Hydrol and methomyl were much lower than those of the N strain.

HAMA and IWATA (1971) found a good correlation between the insensitivity of AChE and the resistance level of the N strain toward several *N*-methylcarbamate insecticides. The AChE of N was also a few times less sensitive to carbanolate and Hydrol than that of the S strain (HAMA and IWATA, 1971). Consequently, it is considered that the resistance to the former group of carbamates, e.g. propoxur, Tsumacide, carbaryl, Bassa and Knockbar, is caused mostly by the insensitivity of AChE, whereas the resistance to the latter group, e.g. carbanolate, Hydrol and methomyl, is probably little related to the alteration of AChE.

The Rmc strain remained moderately resistant to most of the organophosphate insecticides tested, and its dosage-mortality relationships with some organophosphates were not linear (Fig. 7). These results are possibly due to some additional resistant factor(s) remaining in the strain. However, AChE of the Rmc was evidently several times less sensitive to malaoxon, fenitrothion-oxon and chlorfenvinphos than that of strain S (Table 4). In contrast, susceptibility of the Rmc to pyridafenthion, propaphos and acephate was as high as that of S strain, and the sensitivity of its AChE to diazoxon, pyridafenthion-oxon and propaphos did not diminish. These results suggest that the alteration of AChE contributes to some extent in resistance to certain organophosphates such as malathion, phenthoate, fenitrothion and Fujithion, but is not involved in resistance to pyridafenthion, propaphos, acephate and diazinon.

As described in the Introduction, resistance levels of the multi-resistant leafhopper to organophosphate insecticides such as malathion, methyl parathion and fenitrothion were intensified after the occurrence of carbamate-resistance (IWATA and HAMA, 1971). It is suggested that the increase in resistance levels to these organophosphates is due to selection of the gene conferring insensitivity of AChE, as well as to an increased activity for detoxication.

It is noteworthy that diazoxon, pyridafenthion-oxon and propaphos inhibited the altered AChE of the Rmc strain more strongly than that of S strain (Table 4). This finding suggests that the resistant leafhoppers containing the altered AChE might be controlled by compounds of this type.

In vitro inhibition of AChE of both S and Rmc strains by propaphos and acephate was extremely low (Table 4). However, it was observed that AChE activity of the paralyzed or immobilized leafhoppers applied topically with propaphos or acephate was severely inhibited below 30% of the control in both S and Rmc strains (HAMA, 1975, unpublished data). It appears likely that a conversion into an active form is involved in the toxication of these compounds.

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