Electrophoretic Esterase Patterns in the Brown Planthopper, *Nilaparvata lugens* STÅL (Hemiptera: Delphacidae) Which Developed Resistance to Insecticides¹

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Zymograms of protein, AliE and AChE of the susceptible (LE) and the resistant (Rm, Rf, M and Sh) strains of *Nilaparvata lugens* STÅL were detected by disk electrophoresis method. Zymograms of protein were separated into eighteen bands (Pro-1, -2, ..., and -18). The number of bands and migration rates were the same in each strain, but the resistant strains showed remarkably higher activities of Pro-4, -8, -11, -12 and -13 bands than those of the LE strain. The AliE were separated into two bands of E-1 and E-2. The resistant strains showed remarkably higher activities of E-1 and E-2 bands than those of the LE strain. The AChE were separated into eight bands (C-1, -2, ..., and -8). C-4 and C-8 bands were not detected in the LE strain. However, the activity of C-3 band in the Rf strain was lower than that of the LE strain. The relative migration rates of Pro-11, -12 and -13 bands were the same as E-1 and C-8 bands. Therefore the suggestion was made that the activities of these proteins might have some importance in the resistance mechanism of *N. lugens*.

INTRODUCTION

In autumn of 1966, it was announced that the efficacy of organophosphorus insecticides against the brown planthopper, *Nilaparvata lugens* STÅL was lower than when these insecticides were first used in Japan. As a result the insect is becoming the most serious pest on paddy rice in the country. The seasonal fluctuation of a lethal dose to organophosphorus and carbamate insecticides of *N. lugens* was investigated from 1967 to 1977 in Kagawa Prefecture (OZAKI, 1978; OZAKI and KASSAI, 1982). Maintaining effective control techniques for the resistant populations of *N. lugens* is most important. The resistance of *Nephotettix cincticeps* UHLER and *Laodelphax striatellus* FALLÉN to insecticides appeared long ago and many papers have reported the resistance problem with these pests. However, few reports exist on the insecticide resistance of *N. lugens*. This study was made to assay the zymograms of protein, AliE and AChE, and to examine the difference in the activities of the susceptible and resistant strains of this insect.

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MATERIALS AND METHODS

The following strains of *N. lugens* were used: 1) Susceptible (LE) strain: This strain was one selected with low activity of AliE from a Kochinagano population which was collected in 1973 (OZAKI and KASSAI, 1982). 2) Malathion- and fenitrothion-resistant strains (Rm and Rf): The population was collected in the field in Kagawa Prefecture in 1975, and was treated continuously with malathion or fenitrothion for seven generations, producing 70 to 75% mortality in each generation. These surviving were reared for two additional generations without any insecticidal pressure. 3) Mitoyo and Shozu strains (M and Sh) were collected in the fields in the Mitoyo and Shozu areas of Kagawa Prefecture in July 1976. These strains had not yet been treated with insecticides in the field that year. They were reared for two generations in the laboratory without insecticidal pressure.

Estimation of protein and esterase activity: Female adults of each strain were used for determining the protein and esterase patterns throughout this study. The body samples for biochemical experiments were divided into two parts (head to thorax, and abdomen) in a frozen condition. The head to thorax parts were homogenized with 2 μ l per part of tris-buffer (1/10 M, pH 7.5) which included 17% sucrose using a Potter-Elvehjem glass homogenizer under a cool condition. A total of 200 body sample parts of each strain were homogenized each time. The homogenate was briefly centrifuged at 8,000 g for 10 min and the resulting supernatant was used as an enzyme source.

Protein and esterase were separated by disk electrophoresis method using 15% polyacrylamide gel (ORNSTEIN and DAVIS, 1962). One milliliter of fine gel solution was poured into a small glass tube, 0.5 cm in diameter and 7 cm in length, with both ends opened. After solidification of the gel solution, crude gel solution was poured on it to a 1.0 cm depth. After the solidification of the crude gel solution, 0.02 ml of the enzyme source was added, and then the tube was again filled with crude gel solution.

Electrophoresis was carried out in a cool chamber at 5°C. Tris-glycine buffer solution (43 mM, pH 6.8) was used as the pole stock solution. The electrophoresis was carried out at 3 mA per tube for 90 min. After electrophoresis, the gels were removed from the glass tubes.

Activities of protein, AliE and AChE were detected as follows: Protein zymogram: The gel was incubated with detector solution, 1.0 g of amido black 10 B, 10 ml of glacial acetic acid, 50 ml of methyl alcohol and 40 ml of distilled water, for 24 hr at 37°C, and then it was discolored with 7% of acetic acid (OGITA and NAKAMURA, 1967). AliE zymogram: The gel was incubated with 20 ml of phosphate buffer solution (1/20 M, pH 6.8) containing 0.5 ml of 1% acetone solution of β -naphthyl acetate and 20 mg of fast violet B salt for 20 min at 37°C, and then it was rinsed with tap water (OGITA and NAKAMURA, 1967). AChE zymogram: The gel was incubated with 20 ml of phosphate buffer solution (1/20 M, pH 6.8) containing 10 mg of NBT (nitro blue tetrazolium), 20 mg of acetylthiocholine iodide and one milliliter of ethyl alcohol for 24 hr at 37°C and then was rinsed with tap water (OGITA and NAKAMURA, 1967). The activity of protein or esterase band was detected with a Toyo electric densitrol (Toyo Chemical Ind. Ltd.). A 630 nm filter for the protein zymogram and a 505 nm filter for AliE and AChE zymograms were used.

Measurement of resistance level was as follows: LD₅₀ values of *N. lugens* to insecticides

ticides were assayed by a topical application method using three to five-day-old female adults. A technical grade of each insecticide was used and was diluted with acetone. The acetone solution of 0.25 μ l of several concentrations of insecticide was applied to the abdomen of female adults. Treated insects were transferred to a glass tube containing rice seedlings and kept at 25°C. Mortality counts were made 24 hr after treatment.

RESULTS

LD₅₀ values and the resistance levels against carbaryl, MTMC (tsumacide), malathion, fenitrothion, fenthion, diazinon and propathos of *N. lugens* are shown in Tables 1 and 2. The LE strain showed high susceptibility to the test insecticides and the levels were almost the same as those of the Chikugo strain in 1966 (FUKUDA and NAGATA,

Table 1. Insecticidal toxicities to LE, Rm, Rf, M, Sh and Chikugo strains of *Nilaparvata lugens* STÅL

Insecticide	LD ₅₀ value ^a (μ g/g body weight)					
	LE	Rm	Rf	M	Sh	Chikugo ^c
Carbaryl	0.53	2.43	2.29	1.76	2.21	0.67
MTMC	1.24	4.10	— ^b	2.28	3.30	1.83
Malathion	4.42	84.3	71.8	12.0	8.31	6.85
Fenitrothion	7.79	71.4	231.2	66.8	42.1	9.56
Fenthion	1.72	15.1	—	20.4	9.3	8.35
Diazinon	5.93	40.4	35.2	25.9	20.9	7.34
Propaphos	1.54	10.1	—	5.09	4.85	—

^a Female adults were treated by a topical application method and mortality was determined 24 hr after treatment.

^b No test was performed.

LE=susceptible strain, Rm=malathion resistant strain, Rf=fenitrothion resistant strain, M=Mitoyo strain and Sh=Shozu strain.

^c Refer to FUKUDA and NAGATA (1969).

Table 2. Resistant levels of Rm, Rf, M and Sh strains of *Nilaparvata lugens* STÅL to insecticides

Insecticide	Resistant level ^a			
	Rm	Rf	M	Sh
Carbaryl	4.6	4.3	3.3	4.2
MTMC	3.3	— ^b	1.8	2.7
Malathion	19.1	16.2	2.7	1.9
Fenitrothion	9.2	29.7	8.6	5.4
Fenthion	8.8	—	11.9	5.4
Diazinon	6.8	5.9	4.4	3.5
Propaphos	6.6	—	3.3	3.1

^a LD₅₀ value in each strain/LD₅₀ values in LE strain.

^b No test was performed.

Rm=malathion resistant strain, Rf=fenitrothion resistant strain, M=Mitoyo strain and Sh=Shozu strain.

1969). The Rm and Rf strains were just the early stage of resistance to insecticides; therefore, the resistance level of both strains to insecticides was relatively low. The Rm strain had 19-fold resistance for malathion, and 3- to 9-fold for diazinon, propathos, fenitrothion, fenthion, carbaryl and MTMC. On the other hand, the Rf strain had 30-fold resistance for fenitrothion, and 16-fold for malathion, but the resistance level for diazinon and carbaryl was the same as that of the Rm strain. The M strain, collected in the field in July 1976, had 12-fold resistance for fenthion and 9-fold for fenitrothion. However, LD₅₀ values of the M strain for MTMC and malathion were almost the same as those of susceptible (LE) strain. Resistance levels of the M strain for the other test insecticides were lower than those of the Rm and Rf strains. The Sh strain had 5-fold resistance for fenitrothion and fenthion, however, LD₅₀ values for MTMC and malathion were the same as those of the LE strain. The results of both field strains, M and Sh, against fenitrothion indicated that their susceptibility was considerably decreased compared with that of the Chikugo strain in 1966.

The activity of the protein zymogram separated by disk electrophoresis method is shown in Fig. 1. The protein of *N. lugens* was separated into eighteen bands (Pro-1, -2, . . . , and -18). Activities of these bands in the LE strain were lower than those of the Rf and M strains. The same number of protein bands was detected in the Rf, M and LE strains, but the activities of Pro-8, -11, -12 and -13 bands in the Rf and M strains were remarkably higher than those of the LE strain. Also, Pro-4 band in the Rf and M strains showed higher activity than that of LE strain. Activities of Pro-11, -12 and -13 bands in the Rf strain were too high, therefore these bands were not separated into individual bands as was the LE strain. It was not clear whether this high activity was caused by all these bands or only some of them. As shown in Fig. 1, the activity in the M strain was the same as the Rf strain.

The AliE of *N. lugens* was separated into two bands as shown in Fig. 2 (E-1 and E-2).

Fig. 1. Zymogram pattern of protein of *Nilaparvata lugens* STÅL separated by disk electrophoresis method. LE=susceptible strain, Rf=fenitrothion resistant strain and M=Mitoyo strain (upper: activity curve by electric densitrol) (lower: diagram of electrophoretic band).

Fig. 2. Zymogram pattern of AliE of *N. lugens* STAL separated by disk electrophoresis method. LE=susceptible strain, Rf=fenitrothion resistant strain, M=Mitoyo and Sh=Shozu strain (upper: activity curve by electric densitrol) (lower: diagram of electrophoretic band).

Fig. 3. Zymogram pattern of AChE of *N. lugens* STAL separated by disk electrophoresis method. LE=susceptible strain and Rf=fenitrothion resistant strain (upper: activity curve by electric densitrol) (lower: diagram of electrophoretic band).

Activities of both bands in the LE strain was low. The Sh strain had higher activity of the E-1 band and remarkably higher activity of the E-2 band than the LE strain. The M strain was remarkably higher in activity of both E-1 and E-2 bands than the LE strain. The activities of E-1 and E-2 bands in the Rf strain were higher than those of the M strain. AliE in the Rm strain is not presented here, however, it was the same as the Rf strain.

The zymograms of AChE in the LE and Rf strains are shown in Fig. 3. AChEs were separated into eight bands (C-1, C-2, . . . , and C-8), but C-4 and C-8 bands were not detected in the LE strain. The activity of C-3 band in the Rf strain was lower than that of the LE strain.

Comparing the zymograms of protein and AliE, the relative migration distance of E-2 band and Pro-14 band, E-1 and Pro-11, -12 and -13 bands were respectively identical.

Comparing the migration rate of AChE with that of protein or AliE, the C-8 band was located at the same position as those of Pro-11, -12 and -13 or E-1 bands. The C-7, -6, -5, -4, -3, -2 and -1 bands were located at the same respective sites as those of Pro-8, -6, -5, -4, -3, -2 and -1 bands.

DISCUSSION

Resistance levels of *Nephotettix cincticeps* UHLER, *Laodelphax striatellus* FALLÉN and mosquitos such as *Culex pipiens pallens*, *Cx. P. quinquefasciatus* and *Cx. tarsalis* against organophosphorus insecticides were correlated to AliE activity (OZAKI and KOIKE, 1965; OZAKI and KASSAI, 1970; YASUTOMI, 1970; GEORGHIOU and PASTEUR, 1978). The Rm and Rf strains of *N. lugens* showed 15-fold resistance and above to malathion and fenitrothion, and M and Sh strains showed 5-fold resistance for fenthion and fenitrothion. These strains showed remarkably higher activities of E-1 and E-2 bands of AliE zymogram than the susceptible (LE) strain. It may be suggested that the resistant levels of *N. lugens* to organophosphorus insecticides are correlated with AliE activity.

The major factor for the resistant mechanism of *Tetranychus urticae* KOCH against organophosphorus insecticides and *N. cincticeps* against carbamate insecticides was thought to be modified AChE which showed reduced susceptibility to insecticides (SMISSAERT, 1964; HAMA and IWATA, 1971). AChE of *N. lugens* were separated into eight bands and the Rf strain was found to have the activity of C-4 and C-8 bands; however, these bands were not detected in the LE strain. But the activity of C-3 band in the Rf strain was lower than that of the LE strain. There were some differences in the pattern of AChE zymogram and in the activity of each AChE band between resistant (Rf) and susceptible (LE) strains. The biochemical relationship between the activity of each band of AChE and the resistant level to the insecticide was not determined. The role of these AChE bands in the insecticide resistance of *N. lugens* should be clarified in further study.

Comparing the migration rate of AChE with that of protein or AliE, the C-8 band was located at the same position as those of Pro-11, -12 and -13 or E-1 bands. OZAKI and KOIKE (1965) thought that the hydrolysis of β -naphthyl acetate of *N. cincticeps* was caused by both AChE and AliE. The authors believe these proteins which were separated as Pro-11, -12 and -13 bands have some hydrolysing activity against acetylthiocholine iodide. Therefore, it is necessary to study the biochemical character of these proteins.

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