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DEVELOPMENT OF INSECTICIDE RESISTANCE OF BROWN PLANTHOPPERS IN CENTRAL TAIWAN 【Research report】

在台灣中部褐飛蝨抗藥性之發展情形【研究報告】

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Abstract

Since 1976, the toxicity of 14 insecticides to brown planthoppers collected from rice field in central Taiwan has been periodically monitored by a residual film method. LC50 values separately obtained in 1977-1983 and in 1976 were compared to determine if insecticide resistance had developed during these years as a basis upon which to make a refernce to changes in the resistance of brown planthoppers during the corresponding period. The resistance of brown planthoppers to the test insecticides remained relatively unchanged until 1979. In 1980, threir reative resistance (Lc50 value compared with that in 1976) to carbaryl and carbofuran increased 8.4- and 14.8-fold, respectively. Since 1979, their resistance to most organophosphorus insecticides has shown a gradual decrease with he exception of malathion. In 1980, the level of their resistance to malathion and monocrotophos reached a record high. In 1981 and 1982 tests, their resistance to both carbamates and organophosphates decreased gradually except methomyl and malathion. An unusual high-level resistance of brown planthoppers to the 14 test insecticides was found again in 1983, however. To detect the local difference of resistance, a max./min. level of LC50 was employed in 1982. Results shpwed that it varied 2.4- to 15.5-fold in six localities for four of the insecticides tested. In 1983, a similar detection also revealed a 2.4- to 6.2-fold variability. The evidence suggested a local difference. Brown planthoppers were collected in September, October and November of 1983 in six different areas of central Taiwan to measure the monthly changes of insecticide resistance.The monthly changes varied significantly, suggesting that migration was one of the factors that affect-ing the brown planthoppers resistance.

摘要

Key words:

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Development of Insecticide Resistance of Brown Planthoppers in Central Taiwan

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ABSTRACT

Since 1976, the toxicity of 14 insecticides to brown planthoppers collected from rice field in central Taiwan has been periodically monitored by a residual film method. LC_{50} values separately obtained in 1977-1983 and in 1976 were compared to determine if insecticide resistance had developed during these years as a basis upon which to make a reference to changes in the resistance of brown planthoppers during the corresponding period. The resistance of brown planthoppers to the test insecticides remained relatively unchanged until 1979. In 1980, threir relative resistance (LC_{50} value compared with that in 1976) to carbaryl and carbofuran increased 8.4- and 14.8-fold, respectively.

Since 1979, their resistance to most organophosphorus insecticides has shown a gradual decrease with he exception of malathion. In 1980, the level of their resistance to malathion and monocrotophos reached a record high. In 1981 and 1982 tests, their resistance to both carbamates and organophosphates decreased gradually except methomyl and malathion. An unusual high-level resistance of brown planthoppers to the 14 test insecticides was found again in 1983, however.

To detect the local difference of resistance, a max./min. level of LC_{50} was employed in 1982. Results showed that it varied 2.4- to 15.5-fold in six localities for four of the insecticides tested. In 1983, a similar detection also revealed a 2.4- to 6.2-fold variability. The evidence suggested a local difference.

Brown planthoppers were collected in September, October and November of 1983 in six different areas of central Taiwan to measure the monthly changes of insecticide resistance. The monthly changes varied significantly, suggesting that migration was one of the factors that affecting the brown planthoppers resistance.

Introduction

Resistance to insecticides has been a serious problem in the control of insect pests. Many kinds of originally effective insecticides can no longer be used for the control of resistant species while new ones have yet to be developed. So, how to overcome the pest resistance is urgent.

The brown planthopper, *Nilaparvata lugens*, is one of the major rice insect pests in Taiwan. It sucks the sap of rice plants and transmits virus diseases, often causing severe damage to the rice crop. This insect is presented in paddy fields almost throughout the crop season. Its control has been practiced since 1960 by using organophosphates and carbamates. Its frequent exposure to these insecticides plus long-term selection pressure has resulted in the development of insecticide resistance.

In recent years, the resistance of brown planthoppers to the two groups of insecticides has become obvious in Taiwan (Chung *et al.*, 1982; T.C. Chung, 1983; T.Y. Ku, 1976 and 1977; S.C. Wang, 1983). This study is designed to determine changes in their insecticide resistance by a residual film method. Reported here are yearly changes in their insecticide resistance during the period from 1976 to 1983, local differences, inter-generation changes in LC_{50}, and a correlation between esterase activity and four test insecticides was investigated by spectropotometric and electrophorectic techniques.

I. Annual survey of insecticide resistance

Insecticide resistance of brown planthoppers collected annually from the Wufeng area of central Taiwan, in September since 1976 was monitored by a residual film method. Rice seedlings were treated with insecticides at appropriate concentrations in the test. When the rice seedling were dry, 30-50 adults of brown planthoppers were introduced into each test bottle. Mortality was recorded after 24hr. LC_{50} values of some commonly used insecticides for controlling brown planthoppers collected in 1976-1983 are shown in Table 1.

Table 1. LC_{50} Values and Resistance Ratio of Some Commonly Used Insecticides in Brown Planthopper Control (1976-1983) in Taiwan

Insecticides	LC_{50} (ppm)								
	S	'76	'77	'78	'79	'80	'81	'82	'83
Carbaryl	35.8	82.3	47.2	33.0	124.4	694.2	312.6	56.3	325.6
		(2.3)	(1.3)	(6.9)	(3.5)	(19.4)	(8.8)	(1.6)	(9.1)
Carbofuran	18.9	9.0	27.0	30.2	21.2	133.5	82.4	22.8	42.8
		(0.5)	(1.5)	(1.6)	(1.2)	(7.4)	(4.6)	(1.3)	(2.4)
MIPC	16.0	19.5	10.8	17.7	40.4	41.6	21.8	28.2	40.3
		(1.2)	(0.7)	(1.1)	(2.5)	(2.6)	(1.4)	(1.8)	(2.5)
MTMC	21.0	19.0	11.2	8.5	24.9	71.6	37.1	47.8	81.8
		(0.9)	(0.5)	(0.4)	(1.2)	(4.0)	(1.8)	(2.3)	(3.9)
Malathion	110.0	1550.1	300.4	64.4	1935.5	2025.8	1834.5	2287.9	2690.6
		(14.1)	(1.5)	(1.0)	(17.6)	(18.4)	(16.7)	(20.8)	(24.5)
Monocrotophos	44.0	30.9	33.2	45.1	101.5	300.4	125.7	71.1	202.8
		(0.7)	(0.8)	(1.1)	(2.3)	(6.8)	(2.8)	(1.6)	(4.6)
Vamidothion	169.0	236.7	250.3	156.6	167.3	107.2	118.8	96.3	185.7
		(1.4)	(1.5)	(1.0)	(1.0)	(0.6)	(0.6)	(0.6)	(1.1)

S: Susceptible strains.

( ): Resistance ratio = $\frac{LC_{50} \text{ of field populations in 1976-88}}{LC_{50} \text{ of susceptible strains}}$

The LC_{50} value of carbaryl was 82.3 ppm in 1976, increased up to 694.2 ppm and then decreased to 312.6, 56.3, and 325.6 ppm in 1981, 1982, and 1983, respectively. The LC_{50} value of carbofuran had the same tendency: it was only 9.0 ppm in 1976, increased to 133.5 ppm in 1980, and decreased gradually to 22.8 ppm in 1982.

MIPC, MTMC and vamidothion were included in the same insecticide group because their LC_{50} values to tested brown planthoppers showed a very small variation from 19.5, 19.0 and 236.7 ppm respectively in 1976 to 40.3, 81.8 and 185.7 ppm respectively in 1983. Their consumption was large in Taiwan. The reason why brown planthoppers didn't easily develop resistance to them remained unkown.

The LC_{50} value of malathion in the control of brown planthopper was a special case. It was 1550.1 ppm in 1976, decreased to 300.4 and 64.4 ppm in 1977 and 1978 respectively, and then increased gradually from 1935.5ppm in 1979 to 2690.6 ppm in 1983.

From this survey, we found that it was easier for brown planthoppers to develop resistance to malathion than to the other insecticides tested. The LC_{50} value of monocrotophos was 30.9ppm in 1976, maintained at nearly the same level until 1978, increased to 101.5 and 300.4 ppm in 1979 and 1980 respectively, and then decreased to 71.1ppm in 1982 and 202.8 ppm in 1983. All this proved that the variation of annual insecticide resistance in Taiwan was much more complex than in Japan.

II. Comparison of resistance ratios with green rice leafhopper

Brown planthoppers sucked the juice of the rice plants and directly caused "hopper burn" in rice fields, while green rice leafhoppers transmitted virus disease and indirectly caused a serious damage to rice yields. They were important rice pests in Taiwan. In this study we sought to known the difference of resistance levels between these two species under the same chemical condition. Table 2 shows a comparison of insecticide resistance trends between the two species of rice hoppers in 1976 and 1983. Results of the 1976 survey indicated the resistance ratio of brown planthoppers to seven insecticides (including carbaryl, carbofuran, MIPC, MTMC, malathion, monocrotophos and vamidothion) tested ranged from 0.5- to 14.1-fold; but in 1983, it ranged from 1.1- to 24.5-fold.

Table 2. Comparison of Insecticide Resistance Ratios Between Two Species of Rice Hoppers in 1976 and 1983

Insecticides	Resistance ratio			
	B.P.H.		G.L.H.	
	1976	1983	1976	1983
Carbaryl	2.3	9.1	16	78
Carbofuran	0.5	2.4	15	9
MIPC	1.2	2.5	12	52
MTMC	0.9	3.9	23	27
Malathion	14.1	24.5	93	253
Monocrotophos	0.7	4.7	16	36
Vamidothion	1.4	1.1	159	30

B.P.H. = Brown planthopper
G.L.H. = Green rice leafhopper

In other words, the resistance ratio of brown planthoppers to the seven insecticides tested increased 1- to 7-fold in 1976-1983.

Same surveys indicated that the resistance ratio of green rice leafhoppers to seven insecticides tested ranged from 12-to 159-fold in 1976 and from 9- to 253-fold in 1983. Clearly the development of the resistance ratio of green rice leafhopper was faster than that of brown planthoppers. From Table 2, we could find that the variation of LC_{50} values of all the insecticides for the control of green rice leafhoppers tested in 1976-1983 was small. This suggested that the development of their insecticide resistance might reach a stable status in Taiwan.

Table 2 and Fig. 1 also show that both brown planthoppers and green rice leafhoppers developed a high ratio of resistance to carbaryl and malathion - 9.1 and 24.5 respectively and 78 and 253 respectively in the case of green rice leaf- hoppers.

Miyata and Sakai (1981) reported that the major factors involved in malathion-resistant green rice leafhoppers were an increased activity of malathion-degrading carboxylesterase and a decreased sensitivity of acetylcholinesterase to malathion. The degree to which these two factors played a role in this regard varied with the strains. Take carbaryl resistance for example, the main resistance factor was found to be the reduced sensitivity of acetylcholinesterase to carbamates rather than the metabolism of carbaryl. Miyata, Saito, et al. (1983) reported that the aliesterase activity of malathion-resistant strains of brown planthopper significantly was higher than that of susceptible ones. But the mechanism of carbaryl-resistant strains of brown planthopper was unknown. From the resistance trends of brown planthoppers and green rice leafhoppers to carbaryl and malathion in Taiwan, we found there had the same tendency in Japan.

III. Seasonal fluctuation of insecticide resistance

To understand the increased rate of resistance of brown planthoppers in rice fields, samples were collected from six areas (Tsaotun, Wufeng, Shuangtung, Tsaohu, Peishungkeng and Puli) in September, October and November of 1982 and 1983 and LC_{50} values were determined (Fig. 2).

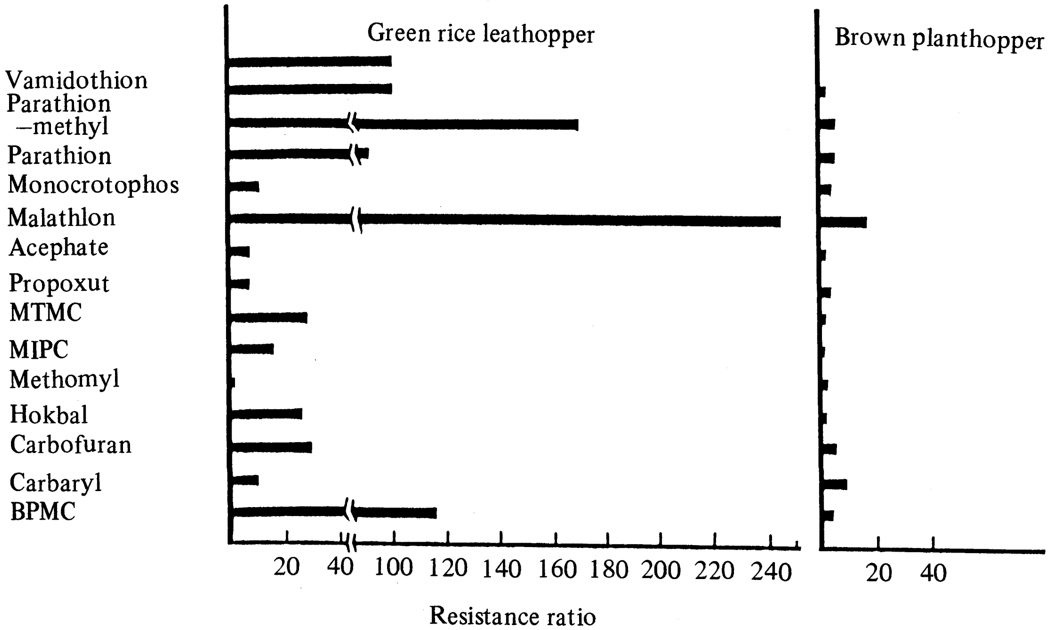


Fig. 1. Resistance ratio of green rice leathopper and brown planthopper to organophosphorus and carhamate insecticides in 1981.

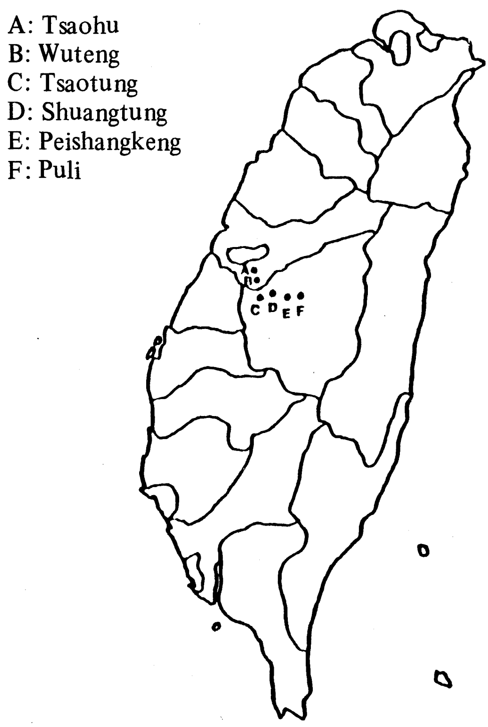


Fig. 2. Location of survey points where brown planthoppers were collected in central Taiwan in 1982 and 1983.

From the above field test, we found that it was easy for brown planthoppers to develop resistance to carbaryl, malathion, carbofuran and monocrotophos. So, we selected these four as representative insecticides in the conduct of experiments.

Table 3 shows a seasonal fluctuation of brown planthoppers resistance to organophosphates in less treated paddy fields at Shuangtung in 1983.

No obvious changes in malathion and monocrotophos resistance was observed between the 1st generation and sucessive generations (2nd and 3rd) in less treated paddy fields with LC_{50} ranging from 2510 to 2589 ppm and 100 to 160 ppm. Table 4 shows the same development trend of resistance to carbamate insecticides such as carbaryl and carbofuran. No obvious change in carbaryl and carbofuran resistance was also observed between the 1st generation and sucessive generations (2nd and 3rd) in less treated paddy fields with LC_{50} ranging from 70-190 ppm and 40-56 ppm.

Table 3. Seasonal Fluctuation of Brown Planthopper Resistance to Organophosphates in Less Treated Paddies in the Shuangtung Area (1983)

Source of strains	LC_{50} (ppm)		Resistance ratio		Date of collection
	Malathion	Monocrotophos	Malathion	Monocrotophos	
1st generation	2589.1	160.2	-	-	Sept. 12
2nd generation	2510.9	100.6	1.0	0.6	Oct. 18
3rd generation	2563.9	144.9	1.0	0.9	Nov. 20

Note: Resistance ratios are calculated on the basis of LC_{50} of the 1st generation.

Table 4. Seasonal Fluctuatuin of Brown Planthopper Resistance in Less Treated Paddies in the Shuangtung Area (1983)

Source of strains	LC_{50} (ppm)		Resistance ratio		Date of collection
	Carbaryl	Carbofuran	Carbaryl	Carbofuran	
1st generation	137.7	50.2	-	-	Sept. 12
2nd generation	190.2	56.1	1.4	1.1	Oct. 18
3rd generation	70.9	40.3	0.5	0.8	Nov. 20

Note: Resistance ratios are calculated on the basis of LC_{50} of the 1st generation.

Table 5 shows a seasonal fluctuation of brown planthopper resistance to organophosphates in treated paddy fields at Wufeng in 1982-1983. The LC_{50} values of malathion and monocrotophos in the 1st generation in 1982 was 57.4 and 24.1 ppm, respectively. Starting from this population, the variation in malathion and monocrotophos resistance in the course of their multiplication in rice fields was traced by checking the insecticide resistance of every subsequent generation in the same fields. The adults of 2nd and 3rd generations which appeared in Oct. and Nov. of 1982, showed an apparent increase in resistance with LC_{50} values of 2064.8 and 2287.9 ppm of malathion and 78.2 and 71.1ppm of monocrotophos, respectively. Thus, the parent population invaded paddy fields by mass production developed 3.6- to 4.0-fold and 3.0- to 3.2-fold, of the resistance to malathion and monocrotophos was noted in only 2nd and 3rd generations after they settled down there. Monocrotophos was applied 6-7 times to the crop in the Wufeng area every year. Malathion, though less used by the farmens, was recommended by the government for aerial spraying to control green leafhoppers.

In 1983, malathion and monocrotophos resistance of brown planthoppers from 1st generation to 3rd generation remained constant. But, there was a small increase of resistance in the 3rd generation.

Table 6 shows a seasonal fluctuation of brown planthopper resistance to carbamate insecticides in treated rice fields at Wufeng in 1982-1983. The resistance of brown planthoppers to carbaryl and carbofuran during the course of their multiplication of the 3rd generation increased 3.9- and 2.5-fold as compared with the 1st generation in 1982. The resistance to the 3rd generation to carbaryl and carbofuran increased 1.3- and 1.8-fold in 1983. From the above results, we could understand brown planthoppers developed resistance 3- to 4-fold only in the 2nd and 3rd generations in heavily treated with the insecticides.

IV. Local insecticide resistance

To undestand the source of brown planthoppers in Taiwan, we used max./min. ratios (Kilin

Table 5. Seasonal Fluctuation of Brown Planthopper Resistance to Organophosphates in Treated Paddies in the Wufeng Area (1982-1983)

Source of strains	LC_{50} (ppm)		Resistance ratio		Date of collection
	Malathion	Monocrotophos	Malathion	Monocrotophos	
1982					
1st generation	574.9	24.1	-	-	Sept. 12, 1982
2nd generation	2064.8	78.2	3.6	3.2	Oct. 10, 1982
3rd generation	2287.9	71.1	4.0	3.0	Nov. 15, 1982
1983					
1st generation	2690.6	202.8	-	-	Sept. 7, 1983
2nd generation	2375.3	191.4	0.9	0.9	Oct. 10, 1983
3rd generation	3911.0	357.3	1.5	1.8	Nov. 16, 1983

Note: Resistance ratios are calculated on the basis of LC_{50} of the 1st generation in the respective year.

Table 6. Seasonal Fluctuation of Brown Planthopper Resistance to Carbamates in Treated Paddies in the Wufeng Area (1982-1983)

Source of strains	LC_{50} (ppm)		Resistance ratio		Date of collection
	Carbaryl	Carbofuran	Carbaryl	Carbofuran	
1982					
1st generation	57.8	9.2	-	-	Sept. 12, 1982
2nd generation	225.4	31.2	3.9	3.4	Oct. 10, 1982
3rd generation	230.6	22.8	3.9	2.5	Nov. 15, 1982
1983					
1st generation	325.4	42.8	-	-	Sept. 7, 1983
2nd generation	201.7	39.5	0.6	0.9	Oct. 10, 1983
3rd generation	409.8	74.9	1.3	1.8	Nov. 16, 1983

Note: Resistance ratios are calculated on the basis of LC_{50} of the 1st generation in the respective year.

and Nagata, 1981)for a comparison of local resistance. Max./min. ratios were maximum LC_{50}/minimum LC_{50}. The data collected in 1982 and 1976 indicated that organophosphates had a remarkable increase in LC_{50}: malathion, 1.2- to 4.8-fold; monocrotophos, 1.0- to 4.6- fold; and carbamate, 1.1- to 4.9-fold. In a comparison of differences in six localities, we found a 2.4- to 15.5-fold variation of insecticide resistance in max./min. ratio of LC_{50} for the insecticides tested (Table 7). The pattern of insecticide resistance of brown planthoppers at Tsaotun and Shuangtung was somewhat different from that in the other four localities, the brown planthopper population Tsaotun exhibited a relatively high resistance to four of the tested insecticides, while the population at Shuangtung showed a low resistance level to four of the insecticides.

A comparison with the data obtained in previous years also showed an increase in LC_{50} values: malathion, 9.4- to 39.8-fold; monocrotophos, 5.2- to 12.7-fold and carbofuran, 4.8- to 29.9-fold. Local differences of insecticide resistance in the six survey areas were higher than in previous years with max./min. LC_{50} ratios increased 2.4- to 6.2-fold (Table 8).

In the case of overwintering insect species, selection pressure by insecticides in each area usually showed remarkable regional differences in insecticide resistance corresponding to the amounts of insecticides used. Local differences of insecticides resistance existed in 1982 and 1983 tests, and the data suggested that brown planthoppers could overwinter in Taiwan. The local difference of insecticide resistance was lesser in 1983 than in 1982. It revealed that part of the brown planthopper populations renewed by migration every year. But the ratio of migration and overwintering is unknown.

V. Insecticide resistance and esterase activity

Ozaki (1969) studied esterase activities of the green rice leafhoppers, *Nephotettix cincticeps* Uhler and the small brown planthopper, *Ladophax striatellus* Fallen. He reported that the esterase bands of hydrolyzed β-naphthylacetate were higher in the malathion resistant strain of these two insect than in the susceptible ones. Since 1975, research has been conducted on the insecticide resistance development of brown planthoppers in Taiwan. To understand their resistance mechanism pattern in rice fields, we ran esterase of brown planthoppers bred in laboratory by a polyacrylamide-gel electrophoresis method. Results showed that the Ali-esterase of brown planthoppers was separated into five bands. (Fig. 3). This seemed to confirm the report of Ozaki (1982). Five esterase bands were detected with the highest activity in the E_2 band of susceptible strains. Table 9 shows the frequency of individuals with a low, medium or high activity of the E_2 band of Ali-esterase of brown planthoppers collected from Tsaotun and Shuangtung in 1982. The samples used were 120 for each area in this test. The esterase activity of the E_2 band of brown planthoppers was 23 at Shuangtung and 37 at Tsaotun.

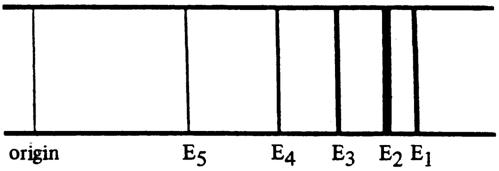


Fig. 3. Schematic representation of zymogram of Ali-esierase (esterase hydrolyzing B–naphthyl acetate) in brown planthoppers.

Table 7. LC_{50} Values for the Fall Generation in 1982

Insecticides	Wufeng	Tsaotun	Shuangtung	Peishankeng	Puli	Tsaohu	Pingtung	Max./Min.[c]	Wufeng (1976)
Carbaryl	230/2.3[a]	275/2.4	128/2.8	129/2.2	177/2.8	317/1.6	176/3.6	2.4	82/2.0
	(2.8)[b]	(3.4)	(1.6)	(1.6)	(2.2)	(3.9)	(2.1)		(1.0)
Carbofuran	23/1.6	33/2.7	25/1.9	17/2.0	10/2.4	18/2.3	44/3.0	4.5	9/2.4
	(2.5)	(3.7)	(2.8)	(1.8)	(1.1)	(1.9)	(4.9)		(1.0)
Malathion	2288/2.0	2875/2.4	182.3/3.6	1491/2.2	802/2.5	1453/1.3	1251/1.2	15.1	155/3.4
	(14.8)	(18.0)	(1.2)	(9.6)	(5.2)	(9.4)	(8.1)		(1.0)
Monocrotophos	71/2.3	142/2.1	31.2/1.9	50/2.2	33/2.0	97/2.9	46/3.3	3.2	30/3.6
	(2.3)	(4.6)	(1.0)	(1.6)	(1.1)	(3.2)	(1.5)		(1.0)

a. LC_{50} (ppm)/slope of regression line.
b. Times of increase of LC_{50} as compared with 1976 data.
c. Max./Min. ratios are maximum LC_{50}/minimum LC_{50} for a local comparison.

Table 8. LC_{50} Values for the Fall Generation in 1983

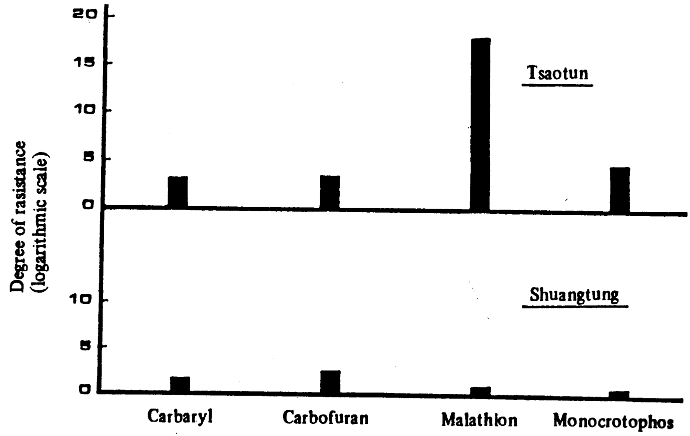
Insecticides	Wufeng	Tsaotun	Shuangtung	Peishankeng	Puli	Pingtung	Max./Min.[c]	Wufeng (1976)
Carbaryl	325/2.6[a]	376/2.6	137/2.3	277/3.3	282/3.3	329/2.3	2.7	82/2.0
	(3.9)[b]	(4.6)	(17.2)	(3.4)	(3.4)	(4.0)		(1.0)
Carbofuran	43/4.2	268/1.6	50/1.3	84/4.3	88/1.5	98/3.5	6.2	9/2.4
	(4.8)	(29.9)	(5.6)	(9.4)	(9.9)	(10.9)		(1.0)
Malathion	2690/5.6	6173/4.3	2589/2.5	2634/3.5	4133/3.2	3089/2.2	4.2	155/3.4
	(17.3)	(39.8)	(16.7)	(9.4)	(26.7)	(19.9)		(1.0)
Monocrotophos	202/2.0	285/2.4	160/1.6	361/2.7	396/4.1	300/2.6	2.4	30/3.6
	(6.6)	(9.2)	(5.2)	(10.3)	(12.7)	(9.7)		(1.0)

a. LC_{50} (ppm)/slop of regression line.
b. Times of increase of LC_{50} as compared with 1976 data.
c. Max./min. ratios are maximum LC_{50}/minimum LC_{50} for a local comparison.

Table 9. Frequency of Individuals with Low, Medium or High Activity of E_2 Band of Aliesterase in 1982.

Population	Esterase activity		
	Low	Medium	High
Shuangtung area	75	22	23
Tsaotun area	56	27	37

The ratio of individuals with a high and medium esterase activity of the E_2 band was 19.1% and 18.3% respectively in the Shuangtung area and 22.5% and 30.8% respectively in the Tsaotun area. In comparing the above esterase activity with the toxicity of brown planthopper population collected from rice fields in Tsaotun and Shuangtung areas, we found that the LC_{50} values of organophosphates and carbamates were slightly higher in the populations at Tsaotun than those at Shuangtung in 1982 (Fig. 4). This suggested a correlation between insecticide resistance and esterase activity.



Fig, 4. Degree of resistance of brown planthopper populations to various insecticides in Shuangtung and Tsaotun areas in 1982.

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