

STUDIES ON CULTIVAR-INDUCED CHANGES IN INSECTICIDE SUSCEPTIBILITY AND ITS RELATED ENZYME ACTIVITIES OF THE WHITE BACKED PLANTHOPPER, *SOGATELLA FURCIFERA* (HORVÁTH) (HOMOPTERA: DELPHACIDAE)

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Abstract The susceptibility of *Sogatella furcifera* female adults on N22 to malathion increased as feeding time prolonged, while the day change of susceptibility to isoprocarb showed a inverse tendency. The activities of esterase and carboxylesterase were induced significantly after on N22 for one day, and then declined. The non-susceptible level of adults to insecticides and enzyme activities seemed to be heightened after feeding on ASD7, a variety resistant to *Nilaparvata lugens*. The total phenol content in rice leaf sheath was the highest in N22, and could be regarded as one of the factors, which caused changes in susceptibility of *S. furcifera* to insecticide after feeding different rice varieties. It was recommended that suitable insecticides and planthopper resistant varieties should be coordinately used in practice so that they contribute more effects respectively.

Key words *Sogatella furcifera*, insecticide resistance, variety

1 INTRODUCTION

The white backed planthopper (WBPH), *Sogatella furcifera* (Horváth), has become one of the most devastating insect pests in rice in many Asian countries, particularly in areas where varieties of resistant to the brown planthopper (BPH), *Nilaparvata lugens* (Stål) have been grown (Heinrichs and Rapusas 1983). Outbreaks of WBPH could lead to severe hopperburn and yield loss of the rice crop if no effective control is achieved. Control of this pest has mainly depended on the application of chemical insecticides, however recent surveys of insecticide susceptibility of WBPH revealed that organophosphorus and carbamate resistances have present since the first half of the 1980's in China (Chao *et al.* 1987, Mao and Liang 1992, Wang *et al.* 1996, Yao *et al.* 2000) and Japan (Endo *et al.* 1988a, Hosoda 1989, Hirai 1994). The resistance mechanism of planthoppers, WBPH, BPH and the smaller brown planthopper, *Laodelphax striatellus*, to or-

ganophosphorus insecticides predominantly involved the increase of in both quantity and activity of esterases (ESTs) or carboxylesterases (CaEs) which related to gene amplification (Ozaki 1969, Hama and Hosoda 1983, Miyata *et al.* 1983, Sun *et al.* 1984, Small and Hemingway 2000, Yao 2001), and the resistance to carbamates involved the susceptibility decrease of acetylcholinesterase (AChE) (Chung and Sun 1983, Hama 1983, Hama and Hosoda 1983; Endo *et al.* 1988b, Yao, 2001). Glutathione S-transferases (GSTs) also probably involved in pyrethroid resistance in *N. lugens* (Hemingway 2000) though GSTs and monooxygenases were once considered to be not important in the resistance of planthopper to insecticides (Chung and Sun 1983, Hama and Hosoda 1983, Miyata *et al.* 1983, Hung *et al.* 1990).

Varietal resistance has been considered to be the most promising strategy in the integrated management of this pest. More than 40 000 rice varieties from the germplasms collected by the Interna-

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tional Rice Research Institute (IRRI) have been screened for resistance to WBPH and about 300 were found to be resistant (Heinrichs and Rapusas 1983). Unfortunately, there are few reports of harmonious control with host plant resistance and insecticides on WBPH, though Heinrichs *et al.* (1984) determined the susceptibility to insecticides of BPH and WBPH when reared on rice varieties with different levels of resistance. Herein we described the results of our study on changes in insecticide susceptibility and the activities of some related enzymes of WBPH adults after feeding on three different rice varieties.

2 MATERIALS AND METHODS

2.1 Treatment of insects

Newly emerged female adults of WBPH, which were reared on rice seedling of TN1 (susceptible variety for 3 generations in the insectary at $(26 \pm 1)^\circ\text{C}$ with a photoperiod of 16:8 h(L:D) were randomly divided into three groups, which were respectively reared with rice seedling (60 days after transplanting) of different varieties, *i. e.*, N22 (moderately resistant to WBPH), ASD7 (moderately resistant to BPH) and TN1 (susceptible). For each group, 600 adults were tested. On the 1st, 3rd and 5th day after treatment, about 200 adults were sampled from their respective varieties and used for bioassays and enzyme assays.

2.2 Bioassays

LD₅₀ values were determined by a topical application method. The insecticides, malathion (organophosphate, 94.5% purity), and isoprocarb (carbamate, 98% purity) were dissolved in acetone with a series of concentrations (5 grades), respectively. Female adults were anesthetized with carbon dioxide, and then a 0.25 μL dose of insecticide solution with a certain concentration was applied to the dorsal thorax by a microapplicator (made by Shanghai Institute of Entomology, Chinese Academy of Science). Control insects were treated with acetone. Each treatment was replicated three times and consisted of 60 adults per replication. Treated females were kept in a glass tube with rice seedlings

(TN1) at $(26 \pm 1)^\circ\text{C}$ in a photoperiod of 16:8 h(L:D). Mortality was recorded after 24 h. LD₅₀ values were calculated by Software DPS® (Tang and Feng 1997).

2.3 Enzyme preparation

An individual adult was homogenized in a glass homogenizer with 100 μL of 0.0067 mol/L phosphate buffer (pH7.2), and centrifuged at 750 *g* for 10 min. The supernatant was used as the source of the enzyme.

2.4 Enzyme assays

EST activity was determined according to the colorimetric method of Hama and Hosoda (1983). Enzyme preparation (5 μL) and 3 mL of the phosphate buffer were added to a test tube and pre-incubated at 37°C , and then 1 mL α -naphthyl acetate with 3×10^{-4} mol/L was added and incubated for 10 min. The reaction was stopped by adding 1 mL of mixture solution of 1% Fast blue B and 5% SDS by the ratio of 2:5. The absorbance was determined at 600 nm.

For the measurement of CaE activity, the phosphate buffer in the reaction system was added eserine (final concentration 10^{-5} mol/L) previously. Other steps were same as the measurement of the esterase activity.

GST activity was determined by the colorimetric method of Booth *et al.* (1961) and Habig *et al.* (1974). The enzyme preparation (20 μL), 2.76 mL of 0.1 mol/L phosphate buffer, pH7.5, 0.2 mL of 0.001 mol/L GSH and 20 μL of 0.1 mol/L CDNB was added. The changes in absorbance at 340 nm within 5 minutes were recorded.

AChE activity was determined by the colorimetric method of Gao (1987). The enzyme preparation (20 μL), 0.9 mL of 0.1 mol/L phosphate buffer, pH7.5, and 100 μL of 5×10^{-4} mol/L acetylthiocholine iodide incubated for 20 min at 37°C , and then 1 mL the following solution was added to stop the reaction. The solution consisted of 12.4 mg DTNB, 120 mL of 96% ethanol and 80 mL H₂O in 0.1 mol/L phosphate buffer (pH7.5), in a total volume of 250 mL. The absorbance at 412 nm was

measured.

Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were measured with disodium phenyl phosphate as the substrate. The enzyme preparation (0.1 mL), 0.5 mL of buffer and 0.5 mL of 0.01 mol/L substrate incubated for 30 min at 37°C, then 1 mL alkaline solution, 0.5 mL 6 g/L 4-amino-antipyrine and 0.5 mL 24 g/L potassium ferricyanide was mixed in turn and the absorbance at 520 nm was measured. The buffer, 0.1 mol/L carbonate buffer (pH 10.0) and alkaline solution (mixture of 0.5 mol/L NaOH and 0.5 mol/L NaHCO₃ by the ratio of 2:3) was used to measure ALP activity, while 0.1 mol/L citrate buffer and the mixture of 0.5 mol/L NaOH and 0.5 mol/L NaHCO₃ by the rate of 1:1 was used to measure ACP activity.

Protein determinations were by the method of Bradford (1976) using bovine serum albumin as standard.

2.5 Total phenol content assays

The total phenol content in rice leaf sheath was measured by the method of Han *et al.* (1993). The

leaf sheath of rice seedling (60 days after transplanting) was homogenized and incubated for 30 min at 80°C, then use Folin method (Swain and Hillis 1959) to determine the absorbance at 700 nm.

3 RESULTS

3.1 Bioassays for malathion and isoprocarb

LD₅₀ of female adults on three varieties to malathion showed that the susceptibilities of WBPH on N22 and ASD7 were little higher though their differences were not significant (Table 1). The day changes in susceptibility of female adults to malathion seemed to show the tendency of increase and then decrease. The LD₅₀ for N22 was about 52.7% those of WBPH on TN1 for 1 day, about 91.0% for 3 days, and 61.3% for 5 days. The value of LD₅₀ of WBPH on different varieties to isoprocarb seemed contrary to those to malathion, which the susceptibility of female adults on N22 and ASD7 were lower, and decreased with feeding time. The LD₅₀ of WBPH on N22 for 3 days response to isoprocarb was about 2.7-fold significantly higher than on TN1.

Table 1 Comparison of insecticidal susceptibility of *S. furcifera* female adults feeding on different rice varieties.

Insecticides	Feeding time(d)	Varieties	LD-P line	LD ₅₀ (μg/mg)	95% Confidence Interval	χ ² (df)*	Body weight (mg/♀)
Malathion	1	TN1	$Y = -3.9608 + 3.3053x$	0.0902	0.0069 ~ 0.1183	0.796(3)	1.424 ± 0.243
		ASD7	$Y = 0.1797 + 1.9567x$	0.0491	0.0024 ~ 0.0997	2.096(3)	1.481 ± 0.097
		N22	$Y = -0.9493 + 2.4173x$	0.0475	0.0285 ~ 0.0794	4.181(3)	1.521 ± 0.109
	3	TN1	$Y = -1.6543 + 2.4053x$	0.0714	0.0408 ~ 0.1248	1.049(3)	1.554 ± 0.353
		ASD7	$Y = -4.7382 + 3.5366x$	0.0877	0.0679 ~ 0.1135	0.116(3)	1.698 ± 0.219
		N22	$Y = -0.7633 + 2.2150x$	0.0650	0.0441 ~ 0.0956	4.519(3)	1.539 ± 0.234
	5	TN1	$Y = -1.7730 + 2.4883x$	0.0613	0.0362 ~ 0.1039	0.898(3)	1.535 ± 0.116
		ASD7	$Y = -1.0835 + 2.3207x$	0.8800	0.0545 ~ 0.1420	2.728(3)	1.698 ± 0.075
		N22	$Y = -30.985 + 15.186x$	0.0376	0.0141 ~ 0.1005	0.000(2)	1.558 ± 0.126
Isoprocarb	1	TN1	$Y = 1.4209 + 2.0610x$	0.0095	0.0059 ~ 0.0173	0.911(3)	1.428 ± 0.218
		ASD7	$Y = 0.7734 + 2.2380x$	0.0144	0.0094 ~ 0.0223	2.255(3)	1.338 ± 0.118
		N22	$Y = -3.0360 + 4.1578x$	0.0165	0.0128 ~ 0.0212	0.093(2)	1.299 ± 0.130
	3	TN1	$Y = -4.0075 + 5.5372x$	0.0066	0.0051 ~ 0.0085	7.213(3)	1.596 ± 0.378
		ASD7	$Y = 1.7797 + 1.6389x$	0.0152	0.0085 ~ 0.0272	0.297(2)	1.516 ± 0.096
		N22	$Y = -1.3196 + 3.0585x$	0.0179	0.0117 ~ 0.0275	0.605(2)	1.623 ± 0.339
	5	TN1	$Y = 0.2310 + 2.1778x$	0.0258	0.0165 ~ 0.0403	7.027(3)	1.500 ± 0.148
		ASD7	$Y = -0.1656 + 2.4037x$	0.0221	0.0141 ~ 0.0348	5.241(2)	1.590 ± 0.128
		N22	$Y = -5.4147 + 4.9783x$	0.0205	0.0141 ~ 0.0299	0.051(2)	1.505 ± 0.127

* $\chi^2_{0.05, df=2} = 5.991$; $\chi^2_{0.05, df=3} = 7.815$

3.2 Enzymes assays

The different rice varieties fed by WBPH female adults could obviously affect the activities of some enzymes, *i. e.* EST, CaE and GST(Fig.1). EST activity and its day change were much different after feeding on different varieties. The EST activity of WBPH on TN1 seemed to be lower than on N22 and ASD7, which decreased with the increase of feeding time. After feeding on N22 for 1 day, EST activity was induced significantly, and then decreased with time. The EST activity on ASD7 was

higher significantly than on TN1 since feeding 3 days, which appeared a high peak on 3rd day. The CaE activity and its day change of WBPH on different varieties showed similar tendency with those in EST though the CaE activities was no significant difference on 5th day. The GST activities of WBPH on N22 and ASD7 were significantly higher than on TN1. The day changes in GST activities on N22 and ASD7 showed a same tendency, increased firstly and then decreased, whereas the activity on TN1 decreased with feeding time.

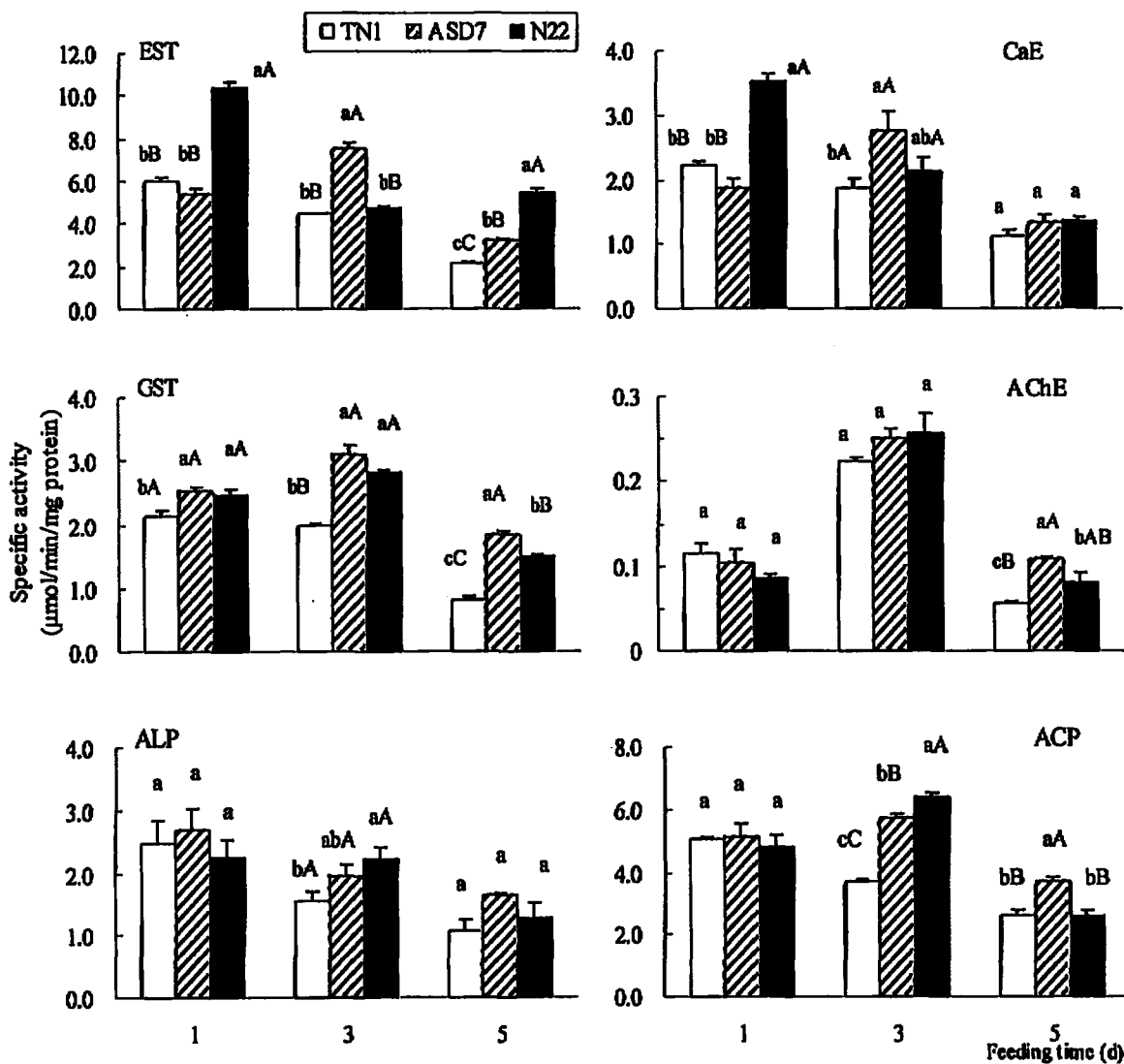


Fig.1 Activity comparison of some enzyme in *S. furcifera* female adults feeding on different rice varieties.

There were no marked differences in AChE activities and their day changes of WBPH on different rice varieties, except by 5th day, the activities of

on N22 and ASD7 were significantly higher than that on TN1. The impacts of rice varieties on ALP of WBPH seemed to be small, whereas the varieties

N22 and ASD7 appeared to have great effects on ACP activities especially in later feeding period.

3.3 Total phenol content assays

The total phenol content in the leaf sheathes of TN1, N22 and ASD7 (60 days after transplanting) were 0.0428 ± 0.0002 , 0.0533 ± 0.0042 and 0.0373 ± 0.0003 mg/g (fresh weight), respectively. ANOVA indicated a significant difference among different varieties ($F = 33.49$, $df = 8$, $P < 0.01$).

4 DISCUSSION

The effects of host plant or allelochemicals on the susceptibility to insecticides and detoxication enzymes of many insects, such as *Spodoptera eridania* (Brattsten and Wilkinson 1977), *S. frugiperda* (Yu *et al.* 1982), *Peridroma saucia* (Berry *et al.* 1980), *Helicoverpa zea* (Muehleisen *et al.* 1989), *Heliothis virescens* (Abd-Elghafar *et al.* 1989), *Melanoplus sanguinipes* (Hinks and Spurr 1989); *Myzus persicae* (Mohamad *et al.* 1989), *Bemisia tabaci* (Omer *et al.* 1993), *Diabrotica virgifera* (Siegfried and Mullin 1989), *Leptinotarsa decemlineata* (Ghidu *et al.* 1990) and *Tetranychus urticae* (Gould *et al.* 1982) were reported. After feeding on different host plants, the insecticide susceptibility of insect pests would decrease, increase or perform no marked change. Different rice varieties fed by WBPH adults also could affect the susceptibility level to insecticides. The susceptibilities of WBPH on N22 or ASD7 to malathion (a kind of organophosphate), and isoprocarb (a kind of carbamate) were much different, which susceptibility to malathion showed a increase tendency while inversely to isoprocarb. The changes in susceptibility to malathion were similar with those reported by Heinrich *et al.* (1984), but inversely to isoprocarb. It suggested that effects of different varieties on the susceptibilities of WBPH to different types of insecticide were much different. The activities of enzymes related to insecticide resistance of WBPH on different varieties changed simultaneously. The activities of EST and CaE of WBPH on N22 for 1 day induced significantly, but decreased following

with feeding time increased, which indicated that host plant shift could impact greatly on the enzyme system of WBPH.

In general, the changes in susceptibility of insect feeding different host plants to insecticides were related to the activation or inhibition of insect detoxifying enzymes induced by plant allelochemicals (Yu 1982, Tan and Guo 1996, Gao *et al.* 1997). As an allelochemical, phenol exists greatly in insect-resistant varieties of rice and wheat (Pathak and Khush 1977, Chen *et al.* 1997). Our results also showed a similar tendency which a high content of total phenol in N22, the variety of mid-resistant to WBPH. Correlation analysis between the contents of total phenol in different varieties and the susceptibility of WBPH on different varieties suggested that there was a high significant negative relationship to malathion susceptibility ($r = -0.574$, $df = 9$, $P < 0.01$), and a positive relationship to isoprocarb susceptibility ($r = 0.126$, $df = 9$, $P > 0.05$), which were probably related to the changes in activities of enzymes of WBPH induced by feeding N22 and ASD7.

The results of this study indicated that the most importance is compatible usage of suitable types of insecticides and planthopper resistant varieties in practice. Thus, both fewer applications of special insecticide and higher mortality of WBPH on resistant varieties would cause insecticide resistance to develop at a slower rate than on susceptible varieties.

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水稻品种诱导白背飞虱药剂敏感性及其相关酶活性变化的研究

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白背飞虱雌成虫取食 N22 后, 对马拉硫磷敏感性随取食天数增加呈增高趋势; 而对叶蝉散的敏感性变化则正相反。在取食 N22 1 天后酯酶/羧酸酯酶即表现高水平的诱导活性, 尔后随取食时间延长而降低。取食抗褐飞虱品种 ASD7 后, 其体内酶活及对药剂抗性多呈升高趋势。不同水稻品种中总酚含量以 N22 显著为高, 相关分析表明, 水稻叶鞘中的酚类物质可能是品种影响飞虱药剂敏感性的主要因素之一。因此, 在生产实践中, 适宜的杀虫剂与飞虱抗性品种应协同使用, 以充分发挥各自的有效作用。

关键词 白背飞虱 抗药性 品种

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