Rice Science, 2004, 11(3): 135 – 139
http://www.ricescience.info

Relationship Between the Development of Methamidophos Resistance and the Activities of Three Detoxifying Enzymes in Brown Planthopper, *Nilaparvata lugens*

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Abstract: Methamidophos resistance of brown planthopper(BPH), *Nilaparvata lugens* was selected in laboratory for 19 generations (F_1 to F_{19}). The resistance development in BPH was approximatively shaped as the letter "S": resistance change was small before the fifth generation and after the fifteenth generation, and the changing pattern was sharp between the fifth and the fifteenth generation. Esterase might play an important role in the resistance development, because the esterase activity and the number of individuals with high activities increased along with the resistance development. The esterase activities of insecticide-sensitive population S, field population F_0, its selective generations F_5, F_{10} and F_{15} were highly correlated with the resistance ratios of these generations, and the coefficient was 0.9899. Mixed-function oxidases and glutathione S-transferase also might play some roles in the resistance development, but the big change in the activities of the two detoxifying enzymes both took place before the tenth generation.

Key words: *Nilaparvata lugens*; methamidophos; resistance to insecticide; detoxifying enzymes

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a major insect pest of rice in many parts of Asian rice growing regions [1]. Chemical control was thought to be the best method for controlling BPH [2] and the extensive use of insecticides has developed the resistance in populations of this pest in different countries and areas. In 1969, Nagata et al. reported the first documented case of insecticide resistance in BPH to BHC [3]. In China, methamidophos has become one of the most important insecticides for controlling BPH. From 1980s, the susceptibility of BPH to methamidophos declined gradually, and in 1995, the resistance ratio was over ten times [4,5]. The mechanisms of insecticide resistance in BPH were very complicated, but metabolic mechanism was considered as one of the most important ones [6]. Esterase, GSH S-transferase (GSTs) and P450 monooxygenases (viz. mixed function oxidase, MFO) were the most important detoxifying enzymes in the insecticide resistance caused by metabolic factors [7,8]. In this paper, we studied the courses of the development of BPH resistant to methamidophos by determining the changes in the activities of three detoxifying enzymes and the number of individuals with different activities.

MATERIALS AND METHODS

Insect

The susceptible strain(S) of BPH was an insecticide-susceptible strain obtained from Jiangsu Academy of Agricultural Sciences in April 2000, rearing in laboratory excluding contact with any insecticide. The field population (F_0) was collected from a paddy field of hybrid rice in Jiangpu, Jiangsu, in July 2000, which was the original strain for resistance selection. The resistance selection was carried out for 19 generations (F_1–F_{19}).

Chemicals

Methamidophos from Bayer with the purity of 98.2%, provided by Professor Toru Nagata (Ibaraki University, Japan), was used in topical treatment and biochemical analysis. Methamidophos in 72.5% used for resistance selection was provided by Suzhou Chemical Company. The following chemicals were used in the experiments: α-naphthyl acetate (α-NA), 1-chloro-2,4-dinitrobenzene (CDNB, Shanghai First Chemical Company); fast blue RR salt (Fluka); glutathione (GSH), $NADPHNa_4$, *p*-nitrophenyl aether (*p*-NA) (Sigma).

Insect bioassay

The bioassay followed the micro-topical application technique reported by Nagata[2]. Macropterous female adults of 3–5 days old were employed as test insects in this study. A drop of 0.0403 μL acetone solution of insecticides was applied topically to the dorsal surface of the thorax of each female that had been anesthetized with carbon dioxide using a hand microapplicator (Burkard Manufacturing Co. Ltd, Rickmansworth, England). Thirty insects were treated at each

Received: 24 December 2002; Accepted: 3 March 2003

concentration, and every treatment was repeated 3 times. Acetone alone served as control used instead of insecticide solution. The treated insects were reared on the seedlings cultured without soil in the rearing cup, at 25±1℃, 16 h light/8 h dark. The mortalities of insects were checked at 24 h after treatment. The LD-*p* line and LD_{50} were calculated and the resistance ratio (RR) was calculated as follows: RR=LD_{50} of any strain or generation / LD_{50} of susceptible strain.

Resistance selection

Resistance was selected by spraying insecticides on the seedlings infested with BPH as described previously[9]. The seedlings cultured without soil were placed in the selection cage (28 cm × 28 cm × 43 cm) and 100–200 3rd-instar larvae were placed in the cage. At 2 h after release, the insecticide in about LC_{60} was sprayed on the seedlings with insects using the pocket sprayer (Hongxing Company, Zhejiang Province). The cages were incubated in an observing room at 28±1℃ and 16 h light/8 h dark. At 3 days after treatment, the surviving insects were transferred into another rearing cage.

Determination of esterase activity

One macropterous female adult in 1–2 days old was homogenized in a glass homogenizer with 0.5 mL of 0.02 mol/L phosphate buffer (pH 7.0) as followed by Han et al [10]. The homogenate was centrifuged at 4 000 r/min and 4℃ for 15 min. The supernatant was utilized as the source of the esterase. In a well of the microplate, 100 μL of the supernatant was put, followed by addition of 100 μL of mixed solution of 2 mmol/L α-NA and 1.5 mmol/L Fast Blue RR Salt. The esterase activity was measured at 450 nm on the Microplate Reader (MODEL 550, BIO-RAD). There were 20 replicates for each generation.

Determination of GSTs activity

One macropterous female adult of 1–2 days old was homogenized in a glass homogenizer with 1000 μL of 0.1 mol/L Tris-HCl buffer (pH 8.0, including 10 mmol/L GSH) using the method of Kao et al [11]. The homogenate was centrifuged at 10 000 r/min for 15 min at 4℃. The supernatant was used as the source of the GSH S-transferase. 100 μL of the supernatant and 1.4 mL Tris-HCl buffer (0.1 mol/L, pH 8.0) were kept in a cuvette at 25℃ for 5 min, and then 60 μL CDNB (30 mmol/L, diluted in acetone) added into the cuvette. GSH S-transferase activity was measured at 340 nm on a 752 UV-Visible spectrophometer. There were 20 replicates for each generation.

Determination of MFO activity

One macropterous female adult of 1–2 days old was homogenized in a glass homogenizer with 2 mL of 0.2 mol/L phosphate buffer (pH 7.8) as described by Hung et al [12]. The homogenate was centrifuged at 10 000 r/min and 4℃ for 15 min. The supernatant was employed as the source of the MFO. 1 mL of the supernatant, 0.5 mL NADPH (1 mmol/L), 0.1 mL *p*-NA (0.1 mmol/L) and 2.5 mL phosphate buffer were introduced into one cuvette, and the cuvette was kept surging on the shaker (THZ-82) at 34℃ for 30 min, and then 1 mL of 1 mol/L HCl was added to cease the action. 5 mL ether and 5 mL chloroform were added into the cuvette successively, and 3 mL solution from the chloroform layer was transferred into another cuvette and 3 mL of 0.5 mol/L NaOH was added into the latter cuvette. The solution from the NaOH layer was used to measure the MFO activity at 400 nm on a 752 UV-visible spectrophometer. There were 20 replicates for each generation.

RESULTS

Changes of methamidophos susceptibility in BPH during the course of resistance selection

The topical LD_{50} values and *b* data for each generation of BPH against methamidophos are given in Table 1 and Fig. 1. The change of LD_{50} value between

Table 1. Changes of the resistance in BPH selected with methamidophos.

Generation	LD-*p* line	LD_{50} (μg/pest)	RR
S	$y = 14.1022+3.8413x$	0.0043	1.00
F_0	$y = 9.1069+2.3958x$	0.0193	4.52
F_1	$y = 8.8235+2.2817x$	0.0211	4.95
F_2	$y = 8.1029+2.0472x$	0.0305	7.14
F_3	$y = 7.9854+2.1306x$	0.0397	9.30
F_4	$y = 7.4775+1.9877x$	0.0567	13.28
F_5	$y = 6.8601+1.9532x$	0.1116	26.13
F_6	$y = 6.1583+1.8476x$	0.2361	55.29
F_7	$y = 5.8663+1.5851x$	0.2841	66.54
F_8	$y = 5.5846+1.7146x$	0.4561	106.82
F_9	$y = 5.3884+1.4917x$	0.5491	128.59
F_{10}	$y = 5.4098+1.6293x$	0.5604	131.25
F_{11}	$y = 5.4029+1.9038x$	0.6143	143.86
F_{12}	$y = 5.3742+1.8740x$	0.6314	147.87
F_{13}	$y = 5.2804+1.8611x$	0.7069	165.54
F_{14}	$y = 5.2138+2.1427x$	0.7947	186.12
F_{15}	$y = 5.1676+2.1692x$	0.8370	196.02
F_{16}	$y = 5.1746+ 2.4247x$	0.8472	198.40
F_{17}	$y = 5.1562+ 2.3160x$	0.8562	200.51
F_{18}	$y = 5.1555+ 2.4441x$	0.8637	202.27
F_{19}	$y = 5.1615+ 2.6123x$	0.8673	203.12

S=Susceptible population; RR= Resistance ratio.

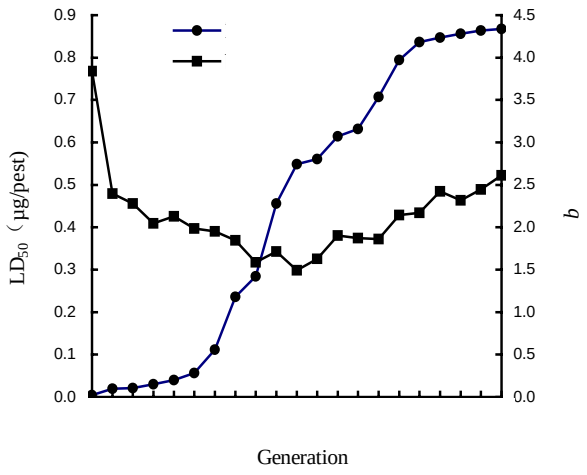

Fig. 1. Changes of LD_{50} and *b* data over the selection generations.
LD_{50} is the dose killing 50 percentage of the population in one test; *b* is the slope of the LD-*p* line.

two successive generations was much different and the resistance development shaped as the letter "S". From the generation F_1 to F_4, the change between two successive generations was smooth at the average increase of 2.19 times. From F_5 to F_{15}, the change was sharp at the average increment of 16.61 times. After F_{15}, the change became smooth again at the average increase of 1.78 times, especially between F_{18} and F_{19} with the increase of 0.85 times. Along with the resistance selection, *b* data decreased firstly with the minimum one of 1.4917 at F_9, and increased gradually afterwards. *b* data of F_0, F_1, F_{18} and F_{19} were 1.51, 1.53, 1.64 and 1.75 times more than that of F_9.

Changes of the activities of three detoxifying enzymes

The activities of the three detoxifying enzymes of F_5, F_{10} and F_{15} are given in Table 2, and the differences between these three generations and S or F_0 were also included. The change in esterase activities was the biggest and highly correlated with the resistance development having the co-efficient of 0.98993 at 0.01 level (t=12.110>$t_{0.01}$= 9.925). The increase of esterase activity of F_5 was relatively lower compared with that of F_0 at the average increase of 0.648 μmol/(min·mg) between two successive generations. After F_5, the esterase activity increased sharply and reached 11.358 μmol/(min·mg) in F_{10} at the average increase of 1.315 μmol/(min·mg) between two successive generations. The rise in esterase activities between F_{10} and F_{15} turned to be lower again, at the average increase of 0.693 μmol/(min·mg) between two successive generations. There weren't significant changes of the activities of MFO and GSTs during the course of resistance selection and the change occurred mainly before F_{10}. If the ratios of the three detoxifying enzymes were multiplied, the product of ratios was high correlated with the resistance development being a co-efficient of 0.99989 at 0.01 level (t=118.653>$t_{0.01}$= 9.925). And the co-efficient between the sum of ratios and the resistance development was also calculated (0.98232), significant at 0.05 level, but not significant at 0.01 level ($t_{0.05}$ = 4.303< t = 9.089 < $t_{0.01}$ = 9.925). The difference between these two coefficients (0.99989 and 0.98232) was investigated and the difference was significant at 0.05 level (t=3.139> $t_{0.05}$=2.776).

Distribution of individuals with different activities of three detoxifying enzymes

Fig. 2 displayed the distribution of individuals with different activities of the three detoxifying enzymes in S, F_0, F_5, F_{10} and F_{15}. There was not considerable difference in esterase activity between F_5 and F_0 with the main range of 0 – 8 μmol/(min · mg). There was noticeable difference between F_5, F_{10} and F_{15}. The main range of

Table 2. Changes of the activities of esterase, mixed-function oxidase (MFO) and glutathione S-transferase (GSTs) in BPH selected with methamidophos.

Detoxifying enzyme		S	F_0	F_5	F_{10}	F_{15}
Esterase	(μmol/min · mg)	0. 951 ± 0. 076 a	1. 543 ± 0. 154 a	4. 781 ± 1. 152 b	11. 358 ± 2. 166 c	14. 825 ± 1. 917 d
	Ratio	1. 00	1. 62	5. 03	11. 94	15. 58
MFO	(pmol/min · mg)	2. 365 ± 0. 307 a	3. 517 ± 0. 422 b	5. 124 ± 0. 750 c	6. 523 ± 0. 816 cd	7. 116 ± 1. 013 d
	Ratio	1. 00	1. 49	2. 17	2. 76	3. 01
GSTs	(μmol/min · mg)	104. 197 ± 7. 442 a	161. 531 ± 13. 675 ab	218. 740 ± 18. 721 b	352. 508 ± 21. 063 c	378. 450 ± 15. 177 c
	Ratio	1. 00	1. 55	2. 10	3. 38	3. 63
Product of ratios		1. 00	4. 20	22. 89	111. 41	170. 27
Sum of ratios		3. 00	4. 66	9. 29	18. 08	22. 23
RR		1. 00	4. 52	26. 13	131. 25	196. 02

Ratio is the result of the division of the activity of one detoxifying enzyme of F_0, F_5, F_{10} or F_{15} by that of S; Different letters in the same row indicate significant difference at 0.05 level.

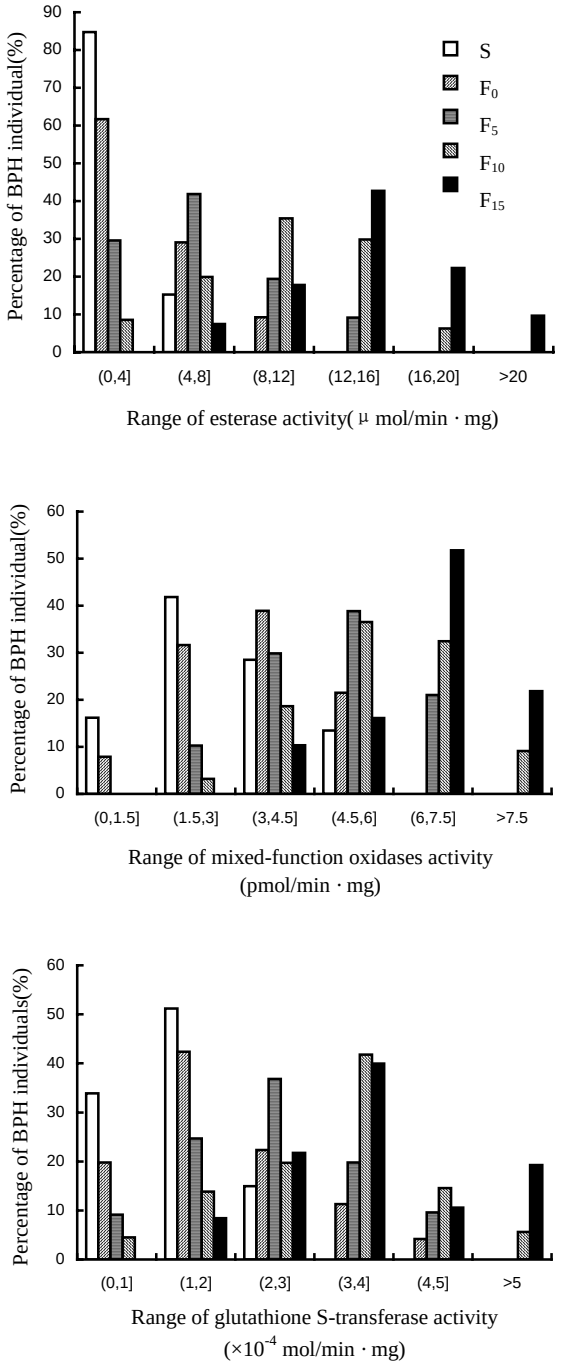

Fig. 2. Distribution of individuals with different activities of the detoxifying enzymes.

F_{10} was 8 – 16 μmol/(min·mg) with 29.83% in 12 – 16 μmol/(min·mg). The main range of F_{15} was 12 – 20 μmol/(min·mg) with 22.33% in 16 – 20 μmol/(min·mg).

In the case of MFO, the distribution was similar in S and F_0. From F_0 to F_{15}, the number of individuals with high activity increased gradually with the main ranges of F_0, F_5, F_{10} and F_{15}[1.5 – 4.5 pmol/(min·mg), 3 – 6 pmol/(min·mg), 4.5 – 7.5 pmol/(min·mg) and >6 pmol/(min·mg), respectively].

Regarding GSTs, there was significant difference between S and F_0 and the distribution of F_0 was broader with some in $(3 – 5)\times10^{-4}$ mol/ (min·mg). There was not much difference between F_0 and F_5 with the main range of $(1 – 3)\times10^{-4}$ mol/ (min·mg). The difference between F_5 and F_{10} was remarkable with the main range of F_{10} at $(3 – 4)\times10^{-4}$ mol/(min · mg). The difference between F_{10} and F_{15} was minor and the distribution was almost same.

DISCUSSION

Along with the resistance selection, the resistance of BPH to methamidophos developed unlinearly in the shape of the letter "S". *b* data declined and then increased after F_9, indicating the change of the homogeneity in the population. In the original field population, the homogeneity was high. The homogeneity declined gradually with the resistance selection, implying that some resistant individuals emerged in the population. The homogeneity exhibited an increasing trend again after F_9, suggesting that the resistant individuals accumulated and many susceptible individuals diminished. Changes in activities of the three detoxifying enzymes in BPH might play some roles in the resistance development to different extents. The investigation on the correlations between the product of ratios and resistance ratio, and between the sum of ratios and resistance ratio revealed that the interrelation between the three detoxifying enzymes was likely the product since the product convincingly interpreted the relationship between the non significant change in the activities of three detoxifying enzymes and the significant change of the resistance. Nevertheless, the product needs to be confirmed through the further studies for some other factors involved in the resistance development. If the hypothesis is confirmed, some small change should be considered regardfully in the studies on resistance mechanism since that change might cause the big development in insecticide resistance.

Esterase played an important role in the organophosphorus insecticide resistance in BPH. In 1969, Ozaki firstly reported that the esterase activity in planthopper individuals resistant to organophosphorus insecticide was higher than that in susceptible individuals to organophosphorus insecticide[13]. Later on, other studies also confirmed that organophosphorus insecticide resistance was correlated with the change in esterase [14,15]. Deng et al. reported that BPH strain resistant to malathion had higher esterase activity and more individuals with high esterase activity than those of susceptible strain [16]. Therefore, our results were very close to their findings. It might be concluded that the increase of esterase activity in BPH was one of the main

reasons causing methamidophos resistance development and at the same time, the other two detoxifying enzymes might accelerate the resistance development.

ACKNOWLEDGEMENTS

This work was supported by the National Key Basic Research Program of China (973 Program)(J20000162). We thank Prof. WANG Yinchang, Nanjing Agricultural University, China for critical reading the early version of the manuscript and his comments. We would like to acknowledge Prof. Toru NAGATA, Ibaraki University, Japan for providing us the easy equipments for rearing the brown planthopper and a microapplicator for topical application. We would extend our thanks to Mr. DONG Zhao and ZHANG Xiaomin in our college for their assistance in BPH rearing and resistance selection.

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