简报 Short Communications

The roles of carboxylesterase and AChE insensitivity in malathion resistance development in brown planthopper

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Abstract: Malathion resistance of brown planthopper (*Nilaparvata lugens* Stål) was selected in laboratory and the successive changes in carboxylesterase (CarE) and AChE were also analyzed. The results showed that the speed of resistance selection varied with generations. The biggest change of LD_{50} occurred between the 3rd – 5th generations. The rise of carboxylesterase activity was correlated with the change in malathion resistance for the first 5 generations, while change in AChE sensitivity between 6th – 8th generations had a higher correlation with the resistance development. Therefore, it was concluded that carboxylesterase activity increase play important role in the early stage of the resistance development and the AChE insensitivity at the late stage.

Key words: malathion; brown planthopper; carboxylesterase; acetylcholinesterase

1 Introduction

The brown planthopper, *Nilaparvata lugens* Stål (BPH), is a major rice pest in many parts of Asia. Extensive use of insecticides has selected for resistance in populations of this pest from different countries and areas (Nagata *et al.*, 1979; Nagata, 1982; Gao *et al.*, 1987). In order to manage resistance, the resistance mechanism had been studied. Esterases played an important role in the resistance of BPH to organophosphorus (OP) insecticides. Ozaki (1969) first reported that OP resistant BPH had higher carboxylesterase activity. Thereafter several papers reported similar results (Hama and Hosoda, 1983; Sun *et al.*, 1984; Tranter and Emden, 1984; Kim and Hwang, 1987; Park and Choi, 1991). The insensitivity of acetylcholinesterase (AChE) may also be important in the OP resistance (Hama and Hosoda, 1983; Park and Emden, 1991). In order to confirm these results and find the roles of different mechanisms in resistance development, we selected BPH with malathion in laboratory and checked the changes in LD_{50}, esterase and AChE at each generation.

2 Materials and Methods

2.1 Insect

The brown planthopper, *Nilaparvata lugens*, used in this study was first collected from the experimental field of Jiangsu, Nanjing. Then was reared on rice seedlings in laboratory, at 25 ± 1℃, 16L/8D.

2.2 Chemicals

Malathion (99.9%) used in the topical treatment and biochemical analysis was provided by Professor Toru Nagata (Ibaraki University, Japan). Malathion (90%) used for resistance selection was provided by Shanghai Pesticide Company. Malaoxon (62.8%) was provided by Jiangsu Pesticide Research Institute. α-Naphthyl acetate (α-NA) was purchased from Shanghai First Chemical Company, fast blue RR salt and acetylcholine iodide (ACHI) from Fluka Chemical Company; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid, from Huamei Biological Engineering Company.

2.3 Resistance selection

Resistance was selected by spraying insecticides on

基金项目:"973"国家重点基础研究项目(J20000162);江苏省科技攻关计划(BE2001345)
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收稿日期 Received: 2002-08-27; 接受日期 Accepted: 2003-01-17

seedlings infested with BPH. The seedling in soilless cultured were placed in the selection cage (28 cm × 28 cm × 43 cm), then 100 – 200 3rd instar larvae were placed in the cage. 2 hours later, the insecticide about LC_{70} in doses was sprayed on seedlings with insects using the pocket sprayer (purchased from Hongxing Company, Zhejiang Province). Then the cage was placed in observing room, at 25 ± 1℃, 16L/8D. More than 2 000 larvae were treated for each generation.

2.4 Bioassay

The bioassay followed the micro topical application technique reported by Nagata (1982). Macropterous adult females of 3 – 5 day-old were used as test animals in this study. A droplet of 0.04 μL acetone solution of insecticides was applied topically to the dorsal surface of the thorax of each female adult that had been anesthetized with carbon dioxide using a hand microapplicator (Burkard Manufacturing Co. Ltd, Richmansworth England). Thirty insects were treated at each concentration, and every treatment was repeated 3 times. Controls used acetone alone instead of insecticide solution. The treated insects were reared on the seedlings soilless cultured in the rearing cage (50 cm × 38 cm × 80 cm), at 25 ± 1℃, 16L/8D. The results were checked in 24 hours.

2.5 Determination of carboxylesterase activity

Ten 3rd instar larvae were homogenized in a glass homogenizer with 1 000 μL of 0.02 mol/L phosphate buffer (pH 7.0) using the method of Hung *et al.* (1990) adapted for use in a microplate reader. The homogenate was centrifuged at 4 000 × g and 4℃ for 30 min, and the supernatant was used as the source of the carboxylesterase. In a well of the microplate, 100 μL of the supernatant was put in, followed by addition of 100 μL of mixed solution of 2 mmol/L α-naphthyl acetate and 1.5 mmol/L Fast Blue RR Salt (containing 10^{-5} mol/L eserine). Then the carboxylesterase activity was measured at 450 nm on the Microplate Reader (MODEL 550, BIO-RAD). Five replications were made for each generation.

2.6 Determination of the I_{50} of malaoxon to AChE

Ten 3rd instar larvae were homogenized in a glass homogenizer with 2 mL of 0.02 mol/L phosphate buffer (containing 0.1% Triton X-100, pH 7.0) using the method of Park and Choi (1991) adapted for use in a microplate reader. The homogenate was centrifuged at 10 000 × g and 4℃ for 30 min, and the supernatant was used as the source of AChE. Six concentrations of malaoxon solution were made by diluting malaoxon using acetone. 5 μL of insecticide solution of each concentration was mixed with 95 μL of the solution of AChE, and the mixture was placed in wells of microplate for 1 hour. The control was set by using phosphate buffer instead of insecticide solution. Then the 100 μL DTNB (300 μmol/L) and 100 μL ATCHI (1.5 mmol/L) were added successively. The residual activity of AChE was measured at 405 nm on the Microplate Reader.

3 Results

3.1 The change of LD_{50} of malathion against BPH and CarE activity after resistance selection

The topical LD_{50} values of malathion against BPH were given in Fig. 1. The LD_{50} of F_0 (the field population unselected) was 0.111 μg/female, which is 10.07 times of S (the susceptible strains in laboratory), which shows that the field population was already with low level of resistance to malathion. From F_1 to F_8, we can find that the change of LD_{50} between two successive generations was different. The change of LD_{50} from F_0 to F_2 was small, and the LD_{50} of F_2 was 0.301 μg/female. The change from F_3 to F_5 was the biggest among the all generations selected, and the LD_{50} of F_5 reached 1.69 μg/female. But the change from F_6 to F_8 became small again, with the LD_{50} of 1.81 μg/female to 2.03 μg/female.

Fig. 1 also showed that carboxylesterase activity increased in successive generations after selection just like the change of LD_{50}. The correlation index between carboxylesterase activity and LD_{50} of each generation reached 0.9929. But from F_6 to F_8 it was only 0.8798. These means that carboxylesterase played an important role in resistance development at the early stage.

3.2 The change of I_{50} of malaoxon against AChE after resistance selection

Fig. 2 showed that the biggest change of I_{50} of malaoxon to AChE did not occur during the generations at which the biggest change of LD_{50} occurred. There was not markable change of I_{50} from F_0 to F_3, and the change was

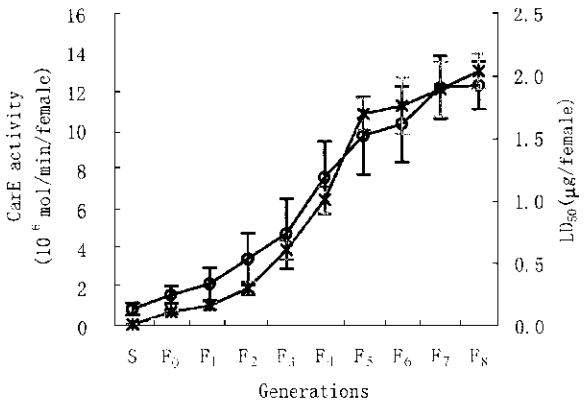

Fig. 1 The change of LD_{50} of malathion against BPH and CarE activity after resistance selection

a little bigger from F_4 to F_5 than that from S_0 to F_3. The biggest change of I_{50} occurred from F_6 to F_8. The correlation index between I_{50} and LD_{50} was 0.8426, and from F_5 to F_8 reached 0.9954. These means the AChE insensitivity play much more important roles in resistance development at the later stage.

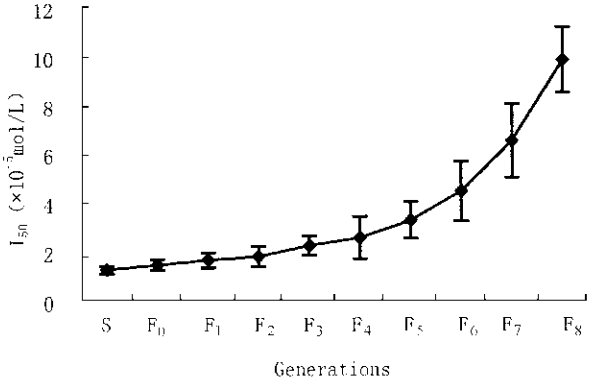

Fig. 2 The change of I_{50} of malaoxon against AChE after resistance selection

Table 1 Three kinetic parameters in S, F_0 and F_8

Parameter	S	F_0	F_8
K_m(10^{-6}mol·mg^{-1}·min^{-1})	5.178 ± 0.746 a	6.242 ± 1.357 ab	8.013 ± 2.146 b
V_{max}(mmol·L^{-1})	1.222 ± 0.104 a	1.340 ± 0.366 a	1.607 ± 0.421 a
K_i(mol·min^{-1})	1.005 ± 0.122 a	1.118 ± 0.205 a	1.694 ± 0.405 b

Notes: Different letters in the same horizontal column showed the significant difference at 0.05 level.

Table 1 showed that there were significant differences between S and F_8 in K_m and K_i and between F_0 and F_8 in K_i. The significantly different K_m between S and F_8 showed that the affinity of AChE in F_8 declined significantly. K_i is one of the parameters for determining the inhibition effect of inhibitors against AChE and the AChE insensitivity. The results showed that the AChE insensitivity in F_8 was significantly higher than that of S and F_0, which indicated that AChE insensitivity might be one of the important mechanisms for malathion resistance in BPH.

4 Discussion

Resistance selection with malathion showed that along with the increase of resistance of BPH, the carboxylesterase activity in the selected strain became higher and the AChE became more insensitive. These results, similar as previous reports, confirmed that both carboxylesterase activity increase and acetylcholinesterase insensitivity played important roles in the OP insecticide resistance of BPH (Hama and Hosoda, 1983).

From the related changes between LD_{50} and carboxylesterase activity or AChE insensitivity in the successive selection generations, it was also found that these two mechanisms played different roles in the resistance development procession. At the early stage, the increase of the carboxylesterase activity was the main cause for resistance quick rise. At the late stage, it was the AChE insensitivity for resistance further growing. These suggested that the metabolizing resistance came first and the target resistance followed in the resistance development. This resistance development mode should be considered in rational pest resistance management.

Acknowledgements We would like to acknowledge the National Key Basic Research Program of China (973 Program, Grant No. J20000162). We also would like to acknowledge Professor Toru Nagata in Ibaraki University of Japan for his provision of the easy equipments for rearing the brown planthopper and Hand Microapplicator.

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羧酸酯酶和乙酰胆碱酯酶在褐飞虱对马拉硫磷抗性发展中的作用

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摘要：对室内筛选褐飞虱 *Nilaparvata lugens* Stål 对马拉硫磷抗性及羧酸酯酶、乙酰胆碱酯酶的连续变化进行了研究。结果表明，抗性发展在不同世代之间存在一定的变化。LD_{50}的最大变化发生在第 3 代和第 5 代之间。在筛选的前 5 代，羧酸酯酶活性上升与马拉硫磷抗性变化存在很好的相关性，而乙酰胆碱酯酶敏感性在第 6 代和第 8 代间的变化与抗性发展存在很好的相关性。可见，羧酸酯酶活性的上升在抗性发展的早期阶段起重要作用，而乙酰胆碱酯酶不敏感在抗性发展的后期阶段起更重要作用。

关键词：褐飞虱；羧酸酯酶；乙酰胆碱酯酶

中图分类号：Q965.9　　**文献标识码**：A　　**文章标号**：0454-6296（2003）02-0250-04