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两个不同生态特征蝗区东亚飞蝗的两种代谢酶

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摘要:黄骅和平山是中国河北省两个不同的蝗区,这两个蝗区的生态特征有很大的不同。黄骅位于河北平原,靠近渤海湾,属于滨海蝗区,蝗区植被以芦苇为主,是我国东亚飞蝗重点防治地区;平山位于河北和山西交界处,仍属于山区,蝗区位于岗南水库库区,属于滨湖蝗区,植被以稗草为主,并兼有玉米、豆类等农作物,为了保护岗南水库水质不受污染,该蝗区很少进行防治。对采自这两个蝗区东亚飞蝗的两种代谢酶:酯酶和谷胱甘肽 S-转移酶进行了比较研究。用对氧磷、马拉氧磷、西维因及毒扁豆碱等 4 种抑制剂对这两个种群飞蝗的酯酶进行体外抑制实验,结果表明,这两个种群的大部分酯酶属于 B-型。在雌性飞蝗中,用 α-NA,α-NB 和 β-NA 3 种酯酶底物测定酯酶活性,黄骅种群比平山种群的酯酶活性分别高 1.63、1.66 和 1.70 倍,雄性中则分别高 1.12、1.41 和 1.27 倍。对两个种群酯酶活性频率分布进行比较,黄骅种群中酯酶活性高的个体数远大于平山种群。两个种群酶活性的差异与马拉硫磷半致死剂量(LD_{50})的差异很相近,这提示酯酶活性的提高在东亚飞蝗对马拉硫磷的抗性中起一定的作用。酯酶活性频率分布显示出东亚飞蝗黄骅种群比平山种群具有较高的马拉硫磷抗性发展趋势,其抗性发展速度较平山种群快。然而,黄骅种群谷胱甘肽 S-转移酶活性略低于平山种群,因此推测,谷胱甘肽 S-转移酶活性与这两个东亚飞蝗种群对马拉硫磷的抗性无明显相关。

关键词:蝗区;酯酶;谷胱甘肽 S-转移酶;杀虫剂抗性;东亚飞蝗

Two metabolic enzymes of oriental migratory locust, *Locusta migratoria manilensis* (Meyen), from two locust areas with different ecological characteristics

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Abstract: Huanghua and Pingshan are two different locust swarming areas in Hebei, China. Huanghua is sea-coast locust area and Pingshan is lake-shored area. Huanghua is a plain in Hebei province close to Bohai bay, where *Locusta migratoria manilensis* (Meyen) feeds on *Phragmites communis* (Trin). Frequent massive outbreaks of *Locusta migratoria manilensis* (Meyen) in Huanghua make it an important area of locust pest control in China. Pingshan is a mountainous area, and collection place is verge of Gangnan reservoir. *Locusta migratoria manilensis* (Meyen) feed on *Echinochloa crusgaii* (L.) Beauv and other crops like corn and legume in Pingshan, and chemicals have been rarely used for locust control for water source protection. General esterases and glutathione S-transferase (GST) of oriental migratory locust, *Locusta migratoria manilensis* (Meyen), collected from the two localities were compared. Inhibition studies of the esterases using four inhibitors (i. e., paraoxon,

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malaoxon, eserine, and carbaryl) indicated that most of general esterases in the two populations were B-type. Using α-NA, α-NB and β-NA as substrates, the activities of esterases were found 1.63-, 1.66-, and 1.70-fold higher for females from Huanghua population and 1.12-, 1.41-, and 1.27-fold higher for males from Huanghua population than those from Pingshan population. Meanwhile Huanghua population had more individuals with high esterase activity than Pingshan population. The difference of LD_{50} value for malathion between the two populations was consistent with the difference of esterase activities, suggesting that general esterases contributed to the malathion resistance of the two populations. The spectrum of esterase activities suggested that Huanghua population had higher potential resistance level than Pingshan population. However, the GST activity of Huanghua population was lower than that of Pingshan population. We suggested that GST activity was not responsible for the malathion resistance of *Locusta migratoria manilensis* (Meyen).

Key words: locust area; esterase; GST; insecticide resistance; oriental migratory locust; *Locusta migratoria manilensis* (Meyen)

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1 Introduction

The oriental migratory locust, *Locusta migratoria manilensis* (Meyen) (Orthoptera: Acrididae) is widely distributed in Southeast Asia (South of N 42°), including Thailand, Cambodia, Indonesia, Japan, China, etc.[1]. The locust species can develop into two phases: the solitary usually of green color and the gregarious yellowish brown color with black stripes on pronotum. In China, the migratory locust occurs in highly divergent ecological habitats. The highest densities of the locust are found in prairies and croplands with abundant food supply. They primarily feed on bulrush and gramineous plants, causing a tremendous losses to gramineous crops because of its huge food consumption and periodic outbreaks[2]. The breeding areas of *Locusta migratoria manilensis* (Meyen) might be divided into four basic types in China, (A) Lake-shored areas, (B) River flood locust areas, (C) Sea-coast locust areas and (D) Inland plain flood areas[1,2]. In recent years, the destructive outbreaks of the oriental migratory locust are increasing in China, both in frequency and scale, possibly due to the global climate changes[2,3]. Huanghua and Pingshan are two important locust areas in Hebei, China with distinct ecological characteristics. Huanghua belongs to sea-coast locust areas, whereas Pingshan belongs to lake-shored locust areas.

Numerous studies have demonstrated that esterases and glutathione S-transferases (GSTs) play important roles in conferring or contributing to insecticide resistance in insect and other arthropod species. In peach-potato aphid (*Myzus persicae*) and southern house mosquito (*Culex quinquefasciatus*), increased carboxylesterase activities are due to the overproduction of unaltered enzymes as a result of gene amplification[4~8]. These enzymes are B-esterases with a very limited ability to hydrolyze the insecticide esters. They cause insecticide resistance primarily via sequestration of insecticides by large amounts of esterases present in resistant insects[8,9]. The GSTs belong to a superfamily that can produce resistance to a range of insecticide by conjugating reduced glutathione (GSH) to the insecticide or its primary toxic metabolic product[10]. GST-based resistance has been demonstrated in *Musca domestica*, *Anopheles gambiae*, *Plutella xylostella* L. and *Blattella germanica et al*[11~13]. However, mechanisms of esterases and GSTs in resistant locust remained unknown.

Malathion is an organophosphate insecticide and has been used for oriental migratory locust control for more than 20 years in China. Recently, however, its efficacy has been observed deceasing in controlling certain populations of the pest[14]. The objectives of this study were to (1) survey the differences of biochemical properties of general esterases and GSTs activity between Huanghua and Pingshan population of the pest and (2) investigate the relationship between the two metabolic enzymes and malathion resistance level in the two field populations.

2 Materials and methods

2.1 Insects

The fifth-instar nymphs of the oriental migratory locust were collected in 2003 from Huanghua (in gregarious phase; namely population-HH) and Pingshan (in solitary phase; namely population-PS), both in Hebei Province, China. All the locusts were stored at −20℃ prior to experiment.

2.2 Chemicals

Bicinchoninic acid solution (BCA), eserine (hemisulfate salt), fast blue B salt (*O*-dianisidine, tetrazotized), α-naphthol,

β-naphthol, α-naphthyl acetate (α-NA), β-naphthyl acetate β-NA), α-naphthyl butyrate (α-NB) were from Sigma Chemical Co. (St. Louis, MO). Paraoxon (90% purity), malaoxon and carbaryl (99%) were from Chem Service (West Chester, PA). Bovine serum albumin (BSA) was from Bio-Rad Laboratories (Hercules, CA). Reduced glutathione (GSH) was from Bio Basic Inc and 1-chloro-2,4-dinitrobenzene (CDNB) was from Sangon.

2.3 Assay of general esterase activity

Whole thorax of fifth instar nymph was homogenized in 0.5 ml ice-cold 0.1 mol/L phosphate buffer (pH 7.5) containing 0.3% (v/v) of Triton X-100. The homogenates were centrifuged at 15 000g for 20 min at 4℃, the supernatants were transferred to clean tubes and used as enzyme sources. General esterase activities were assayed by the method of van Asperen[15] with some modifications by Zhu and He[16] using α-NA, β-NA and α-NB as substrates. General esterase activities were assayed with 31 replicates, each with three determinations.

2.4 In vitro inhibition of general esterases

Inhibitions of general esterases by paraoxon, malaoxon, carbaryl and eserine were determined with females and males of population-HH and population-PS. Paraoxon was diluted with 0.1 mol/L phosphate buffer (pH 7.0) due to its strong hydrolyzing ability. The others were diluted with 0.1 mol/L phosphate buffer (pH 7.5). A group of ten whole locust thoraxes was homogenized as described above. After the homogenates were centrifuged at 15 000g for 20 min at 4℃, the supernatants were transferred to clean tubes and used as enzyme sources. The inhibition reaction was started by incubating 10 μl of the enzyme preparation with 10 μl of each inhibitor at room temperature for 5 min, and terminated by adding of 130 μl of 31.2 mmol/L α-NA. The remaining esterase activity was determined immediately.

2.5 Assay of GST activity

The preparation of the enzyme sources was as in assay of esterase activity. GST activities were assayed by the method of Yang[17] with some modifications using CDNB as substrate. Briefly, 10 μl of enzyme sample were decanted into the wells of a microplate. 190 μl of the CDNB-GSH mixture (10.35 mmol/L CDNB : 200 mmol/L GSH = 188 : 2 (v/v)) was added to each well. The change in absorbance was recorded at 340 nm for 1 min using the microplate reader. GST activities were calculated using the extinction coefficients of 9.6 mmol/(L · cm).

2.6 Protein assay

Protein concentrations of the enzyme preparations were determined according to Smith *et al.* using BSA as a standard[18]. Briefly, 20μl appropriately diluted protein samples or BSA solution were decanted into the wells of a microplate. Add 180μl of the BCA/Cu^{+} solution (BCA solution: 4% cupric sulfate solution = 50 : 1 (v/v)) to the wells containing the protein samples. Incubate the microplate for 30 min at 37 ℃ then set for 5 min at room temperature. The absorbance was recorded with the microplate reader at 560 nm[19].

2.7 Data analysis

All comparisons were subjected to Student's *t*-test using SPSS program.

3 Results

3.1 Esterase activity spectrum

Fig. 1 shows the esterase activities spectrum difference between the two populations using three selected substrates. The frequency of esterase activity for population-HH was in the high activity range, but those of population-PS in the low activity range. Table 1 shows the differences in esterase specific activities between population-HH and population-PS using α-NA, α-NB and β-NA as substrate. No significant differences in

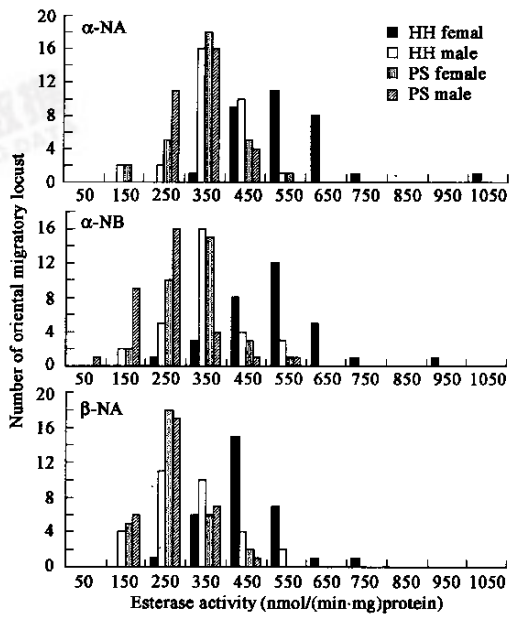

Fig. 1　Esterase activity profiles in Huanghua poulation (HH) and Pingshan population (PS) with the substrate α-NA, α-NB, and β-NA. The esterase activity was individually determined in 31 thoraxes of locust for each sex of each population using the esterase microassay

the esterase activities were detected in male between Huanghua and Pingshan populations using β-NA as substrate and in population-PS between female and male using α-NA as substrate. When α-NA, α-NB and β-NA were used as substrates, females' general esterase activities of population-HH was 1.63-, 1.66- and 1.70-fold higher than those of population-PS and males' general esterase activities of population-HH was 1.12-, 1.41- and 1.27-fold higher than those of population-PS, respectively.

Table 1 General esterase activities (μmol/(min · mg)) **using α-NA, α-NB and β-NA as substrates in Huanghua** (HH) **and Pingshan** (PS) **populations of *Locusta migratoria manilensis*** (Meyen)[a]

Sex	α-NA		α-NB		β-NA	
	HH	PS	HH	PS	HH	PS
♀	0.556±0.125a	0.341±0.083a*	0.528±0.130a	0.318±0.082a*	0.451±0.103a	0.266±0.078a*
♂	0.368±0.077b	0.328±0.067a*	0.358±0.101b	0.254±0.096b*	0.322±0.096b	0.253±0.069b

[a] Results are presented as the mean ± SD; Means within columns followed by the same letter are not significantly different ($P>0.05$) by Student's *t*-test; * Means within rows with the same substrate are significantly different ($P<0.05$) by Student's *t*-test

3.2 *In vitro* inhibition of general esterases

Two organophosphates (paraoxon and malaoxon) and carbamates (eserine and carbaryl) were used in *in vitro* inhibition of general esterases. Paraoxon was the most potent inhibitor of the esterases and eserine was the least potent inhibitor of the esterases (Table 2, Fig. 2), suggesting that most of general esterases in the two populations were B-type based on the classification of esterases by Aldridge[20]. There were significant differences in the pI_{50} (the negative logarithm of the medium inhibition concentration) values for paraoxon and eserine in females, and for carbaryl in male between the two populations by Student's *t*-test (Table 2).

Table 2 pI_{50} values for paraoxon, malaoxon, carbaryl and eserine in in vitro inhibition to esterases of Huanghua (HH) **and Pingshan** (PS) **populations of *Locusta migratoria manilensis*** (Meyen)[a]

Inhibitor	Population	pI_{50}		Regression coefficient, r		r significance, p	
		Female	Male	Female	Male	Female	Male
Paraoxon	HH	6.36±0.11a	6.17±0.14a*	<−0.98	<−0.98	<0.001	<0.001
	PS	6.05±0.04b	6.06±0.16a	<−0.98	<−0.98	<0.001	<0.001
Malaoxon	HH	6.16±0.11a	6.33±0.15a	<−0.99	<−0.93	<0.001	<0.005
	PS	5.76±0.25a	6.44±0.45a*	<−0.98	<−0.97	<0.001	<0.001
Carbaryl	HH	4.29±0.29a	2.90±0.57b*	<−0.98	<−0.98	<0.001	<0.005
	PS	4.07±0.93a	4.59±0.22a	<−0.96	<−0.98	<0.001	<0.001
Eserine	HH	2.72±0.11b	2.65±0.44a	<−0.97	<−0.98	<0.001	<0.001
	PS	3.25±0.17a	2.54±0.37a*	<−0.98	<−0.95	<0.001	<0.01

[a] Results are presented as the mean ± SD ($n=4$); Means within columns with the same inhibitor followed by the same letter are not significantly different ($P>0.05$) by Student's *t*-test; * Means within rows are significantly different ($P<0.05$) by Student's *t*-test

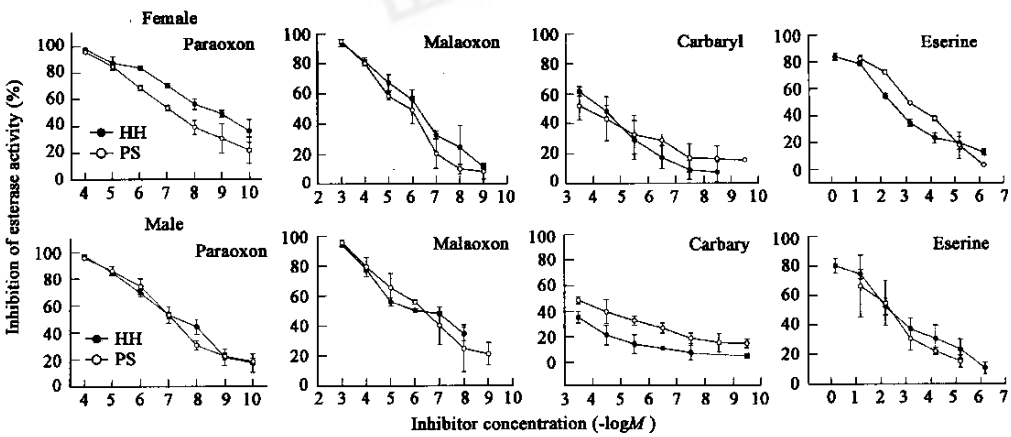

Fig. 2 Inhibition of general esterases from the Huanghua(HH) and Pingshan(PS) populations of *L. migratoria manilensis*(Meyen) by four selective inhibitors:paraoxon, malaoxon, carbaryl and eserine at room temperature(approx. 24℃); Vertical bars indicated SD of the mean of four determinations($n=4$)

3.3 Assay of GST activity

The differences of GST activity of population-HH and population-PS are showen in table 3. The GST activities of population-HH were lower than those of population-PS. The GST activity of population-PS was 1.13 fold as high as that of population-HH in females, and 1.18-fold as high as in males. The GST activities in males were 1.20- and 1.25-fold as high as those in females for population -HH and population-PS.

Table 3　GST activities of *Locusta migradtoria manilensis* (Meyen) collected from Huanghua (HH) and Pingshan (PS) (μmol/(min·mg) protein)

Sex	HH	PS
Female	5.15±1.39a	5.83±1.01a
Male	6.20±1.34b	7.30±1.23b*

[a] Results are presented as the mean ±SD(n=15). Means within columns followed by the same letter are not significantly different(P>0.05) by Student's t-test; * Means within rows are significantly different(P<0.05) by Student's t-test

4 Discussion

4.1 General esterase characterization and relationship to malathion resistance

General esterases are commonly classified into three types according to their interactions with organophosphate compounds (OP)[20]. The A-esterases are not inhibited by OPs but degrade these insecticides as their substrates, whereas the B-esterases are inhibited readily by OPs[21]. The C-esterases, which was later added to the classification, do not interact with OPs[22]. *In vitro* inhibition showed that about 93.0 and 85.8% of general esterases in the females and 87.3 and 78.8% in the males for population-HH and population-PS, respectively, were B-type esterases, and carboxylesterases were predominant in the composition of general esterases in *Locusta migratoria manilensis* (Meyen)[23].

Malathion as an important organophosphate insecticide has been used to control *Locusta migratoria manilensis* (Meyen) in China since 1983. Recently, it has been noted by the pesticide users that malathion control of some populations of the oriental migratory locust in Tianjin became increasingly difficult. They experienced that the oriental migratory locusts in Beidagang, Tianjin had developed 2.9 to 4.5- fold resistance compared with locust of Hangu in Tianjin, which was a relatively sensitive population[14]. Our primary study showed that LD_{50} value of population-HH for malathion (15.09 μg/g insect weight)[23] was very close to that of Beidagang (18.84 μg/g insect weight) (unpublished data). LD_{50} value of population-PS for malathion (8.57 μg/g insect weight)[23] was 1.76, 2.20- fold lower than that of Huanghua and Beidagang, respectively. The ecological characteristics of Huanghua and Beidagang were very similar with salty soils as they are close to Bohai Bay and belong to sea-coast locust areas. *Locusta migratoria manilensis* (Meyen) feed on *Phragmites communis* (Trin) in the two locust areas. The breeding frequencies of locust in Huanghua and Beidagang were similar and they were priority areas of locust control, malathion has been frequently used. Furthermore, population genetic studies of the locusts from Beidagang and Huanghua showed the two populations had similar genetic background[24]. It was proposed that population-HH had similar resistance levels to the locust from Beidagang. However, ecological characteristic of Pingshan is different from Huanghua and Beidagang. Individuals in Pingshan were collected on verge of Gangnan reservoir, where soils are sandy. *Locusta migratoria manilensis* (Meyen) in Pingshan feed on *Echinochloa crusgaii* (L.) Beauv and some crops like corn and legume. Breeding frequencies of locust in Pingshan were once every 3 to 5 years, lower than that in Huanghua and Beidagang[2]. Malathion was sprayed to control locust pest five times in small scale in Pingshan before our specimen collection. We suggest that the higher resistance level of population-HH than population-PS is due to the more severe insecticide pressure to population-HH than to population-PS. A baseline sensitive population of oriental migratory locust is needed to determine the resistant levels of population-HH and population-PS.

Numerous studies have demonstrated that esterases play an important role in conferring or contributing to insecticide resistance in insect and other arthropod species. Zhu *et al*. proposed that αNA-hydrolyzing esterases could be used to accurately reflect the resistance situation of greenbugs (*Schizaphis graminum*) in fields[25]. The insect esterases can either cause broad spectrum resistance to various insecticides through rapid binding and slow turnover of insecticide molecules (i. e., sequestration) or cause narrow spectrum resistance to very restricted range of insecticides containing a common ester linkage such as malathion through rapid metabolism of the insecticides[26, 27]. Between population-HH and population-PS, the difference of resistance level to malathion corresponded to the esterase activity variations. Though general esterase activities of population-HH had only 1.2 to 1.7-fold higher than those of population-PS when α-NA, α-NB and β-NA were used as substrates, the spectrum of general esterase activities showed that individuals with high esterase activity were increased in population-HH using the three selected substrates (Fig. 1). These results suggested that activities of general esterases were

increased along with the resistance to malathion in the two populations. Based on the spectrum of esterase activities of the two populations, it could be inferred that population-HH had higher potential resistance to malathion than population-PS. Since most esterases in population-HH were B-type, we proposed sequestration may be responsible for the increase of esterase activities.

4.2 The role of GST in the insecticide resistance of oriental migratory locust

Increased GST activity was an important mechanism of insecticide resistance in insect. Furthermore, sublethal doses of abamectin and β-cypermethrin could enhance the GST activity in sensitive strain of diamondback moth (*Plutella xylostella* (L.)), but inhibit the GST activity of resistant strain of diamondback moth[28]. GST activity in population-HH was lower than that in population-PS, which was a relatively sensitive population to malathion. This result is consistent with the result obtained by Liang *et al.*[28] since no sensitive locust population with increasing GST activity was available so far, it was inclined that elevated GST activity did not contribute to the malathion resistance in *Locusta migratoria manilensis* (Meyen).

4.3 Implications to the pest control and management

The devastation of swarming locust pest was ofen compared to that of flood and drought in the Chinese history[2]. Controls by manual catching, use of insecticides and through environmental reformation are common practices in locust abatement programs in the country. Chemical control, especially the use of organophosphate compounds, remains to be the major measure. As a result, some locust populations have been noted developing resistance or potential resistance to some chemicals. In this study, we investigated the resistance of population-HH and population-PS to malathion. Our previous studies showed that acetylcholinesterases on which OP insecticides acted, purified from population-HH was 62-, 2.0- and 1.6-fold less sensitive to inhibition by the three OP compounds, chlorpyrifos oxon, demeton-S-methyl and paraoxon, respectively, than that from population-PS[29]. It indicated that the resistance levels of *Locusta migratoria manilensis* (Meyen) to the three OP insecticides were different. We estimated that resistance level for chlorpyrifos of population-HH was much higher than that of population-PS. It is necessary to determine the level and spectrum of OP (i. e. parathion, chlorpyrifos and demeton-S-methyl) resistance in *Locusta migratoria manilensis* (Meyen) from different locations. Based on the spectrum of OP resistance in *Locusta migratoria manilensis* (Meyen), appropriate chemicals could then be recommended for locust control. Since chemical control would inevitably result in the development of insecticide resistance and other unforeseen problems other measures such as bio-control (e. g. application of *Metarhizium*, *Nosema locustae*)[30~33] and environmental modification[2] are preferable alternatives.

References:

[1] Chen Y L. Main achievement of research and management of migratory locust in China. *Entomological Knowledge*, 2000, **37**(1): 50~58.

[2] Zhu E L. *The occurrence and management of oriental migratory locust*. Beijing: Chinese Agricultural Press, 1999.

[3] Chen Y L. The characteristic, cause and ecological management of locust disaster. *Bulletin Biology*, 2000, **35**(7): 1~5.

[4] Devonshire A L. The properties of a carboxylesterase from the peach-potato aphid, *Myzus persicae* (Sulz.), and its role in conferring insecticide resistance. *J. Biochem.*, 1977, **167**(3): 675~683.

[5] Ketterman A J, Jayawardena K G, Hemingway J. Purification and characterization of a carboxylesterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. *J. Biochem.*, 1992, **278**:355~360.

[6] Devonshire A L, Sawicki R M. Insecticide-resistant *Myzus perwesicae* as an example of evolution by gene duplication. *Nature*, 1979, **280**: 140~141.

[7] Devonshire A L, Field L M. Gene amplification and insecticide resistance. *Annual Review Entomology*, 1991, **36**:1~23.

[8] Hemingway J, Karunatne S H P P. Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Med. Vet. Entomol.*, 1998, **12** (1):1~12.

[9] Devonshire A L, Moores G D. A carboxyesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). *Pestic. Biochem. Physiol.*, 1982, **18**: 235~246.

[10] Hemingway J. The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. *Insect Biochem. Mol. Biol.*, 2000, **30**: 1009~1015.

[11] Grant D F, Hammock B D. Genetic and molecular evidence for a *trans*-acting regulatory locus controlling glutathione S-transferase-2 expression in *Aedes aegypti*. *Molecular and General Genetics*, 1992, **234**: 169~176.

[12] Tang Z H, Zhou C L, Wu S C, *et al*. Insecticide resistance and the effects of synergists in the diamondback moth from Shanhai. *Acta*

Phytophylacica Sinica, 1992, **19** (2): 179～185.
[13] Lee C Y, Hemingway J, Yap H H, *et al*. Biochemical characterization of insecticide resistance in the German cockroach, *Blattella germanica*, from Malaysia. *Med. Vet. Entomol.*, 2000, **14**: 11～18.
[14] Yang H J, Wang D S, Zhang L S, *et al*. Preliminary study on the resistance of *Locusta migratoria manilensis* to malathion. *Plant Protec. Technol. Extens.*, 2002, **22**(8): 11～12, 16.
[15] Asperen van K. A study of housefly esterases by means of a sensitive colorimetric method. *J. Insect Physiol.*, 1962, **8**: 401～416.
[16] Zhu K Y, He F Q. Elevated esterases exhibiting arylesterase-like characteristics in an organophosphate-resistant clone of the greenbug, *Schizaphis graminum* (Homoptera: Aphididae). *Pestic. Biochem. Physiol.*, 2000, **67**: 155～167.
[17] Yang X M, Buschman L L, Zhu K Y, *et al*. Susceptibility and detoxifying enzyme activity in two spider mite species (Acari: Tetranychidae) after selection with three insecticides. *J. Econ. Entomol.*, 2002, **95** (2): 399～406.
[18] Smith P K, Krohn R I, Hermanson G T, *et al*. Measurement of protein using bicinchoninic acid. *Anal. Biochem.*, 1985, **150**:76～85.
[19] Zhu K Y, Clark J M. Purification and characterization of acetylcholinesterase from the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Insect Biochem. Mol. Biol.*, 1994, **24**:453～461.
[20] Aldridge W N. The esterases: Perspectives and problems. *Chem.-Bio. Interactions*, 1993, **87**:5～13.
[21] Aldridge W N. Serum esterases Ⅰ. Two types of esterases (A and B) hydrolyzing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. *J. Biochem.*, 1953, **53**:110～117.
[22] Bergmann F, Segal R, Rimon S. A new type of esterase in hog-kidney extract. *J. Biochem.*, 1957, **67**:481～486.
[23] He Y P, Ma E B, Zhu K Y. Characterizations of general esterases in relation to malathion susceptibility in two field populations of the oriental migratory locust, *Locusta migratoria manilensis* (Meyen). *Pestic. Biochem. Physiol.*, **78**(2): 103～113.
[24] Zheng X Y, Duan Y H, Li C X, *et al*. Comparative allozyme analysis of oriental migratory locust *Locusta migratoria manilensis* from two breeding areas in north China. *Acta Genetica Sinica*, 2002, **29**(11): 966～971.
[25] Zhu K Y, Gao J R. Kinetic properties and variability of esterases in organophosphate-susceptible and -resistant greenbugs, *Schizaphis graminum* (Homoptera: Aphididae). *Pestic. Biochem. Physiol.*, 1998, **62**:135～145.
[26] Herath P R J, Hemingway J, Weerasinghe I S, *et al*. The detection and characterization of malathion resistance in field populations of *Anopheles culicifacies* B in Sri Lanka. *Pestic. Biochem. Physiol.*, 1987, **29**:157～162.
[27] Karunaratne S H P P, Hemingway J, Weerasinghe I S, *et al*. Kinetic and molecular differences in the amplified and non-amplified esterases from insecticide-resistant and susceptible *Culex quinquefasciatus mosquitoes*. *J. Biol. Chem.*, 1995, **270**:31124～31128.
[28] Liang P, Xia B, Shi T, Gao X W. Effect of sublethal doses of abamectin and β-cypermethrin on glutathione S-transferases in diamondback moth *Plutekka xylostella* (L.). *J. China Agricultural University*, 2003, **8** (3): 65～68.
[29] Ma E B, He Y P, Zhu K Y. Comparative studies of acetylcholinesterases puritied from two field populations of the oriental migratory locust (*Locusta migratoria manilensis*): implications of insecticide resistance. *Pestic. Biochem. Physiol.*, 2004, **78** (1): 67～77.
[30] Lei Z R, Wen J Z, Tan Z H, *et al*. Field trial with *Metarhizium* oil liquid for controlling *locusta migratoria manilensis* (Meyen). *Plant Protection*, 2003, **29** (1): 17～19.
[31] Zhang L. *Nosema locusta* and its actions in locust control. *Bulletin Biology*, 1999, **34** (2):11～12.
[32] Zhang L, Yan Y H, Shi W P, *et al*. *Locusta migratoria manilensis* prevent and control applying *Nosema locusta* and Cascade^{R}. *Chinese J. Bio. Cont.*, 1999, **15** (2): 57～59.
[33] Hao S G, Qin Q L, Wang Z J, *et al*. Management strategies and control techniques for locust and grasshopper plagues around the world: status and perspectives. *Acta Entom. Sinica*, 2002, **45** (4): 531～537.

参考文献:

[1] **陈永林. 中国的飞蝗研究及其治理的主要成就. 昆虫知识**, 2000, **37** (1): 50～58.
[2] **朱恩林. 中国东亚飞蝗发生与治理. 北京:中国农业出版社**, 1999.
[3] **陈永林. 蝗虫灾害的特点、成因和生态学治理. 生物学通报**, 2000, **35** (7): 1～5.
[12] **唐振华, 周成理, 吴世昌, 等. 上海地区小菜蛾的抗药性及增效剂的作用. 植物保护学报**, 1992, **19** (2): 179～185.
[14] **杨红军, 王东升, 张立顺, 等. 东亚飞蝗对马拉硫磷抗性研究初报. 植保技术与推广**, 2002, **22** (8): 11～12, 16.
[24] **郑先云, 段毅豪, 李春选, 等. 华北 2 蝗区东亚飞蝗种群遗传结构的比较研究. 遗传学报**, 2002, **29** (11): 966～971.
[28] **梁沛, 夏冰, 石泰,等. 阿维菌素和高效氯氰菊酯亚致死剂量对小菜蛾谷胱苷肽 S-转移酶的影响. 中国农业大学学报**, 2003, **8** (3): 65～68.
[30] **雷仲仁, 问锦曾, 谭正华, 等. 绿僵菌油剂防治东亚飞蝗田间试验. 植物保护**, 2003, **29** (1): 17～19.
[31] **张龙. 蝗虫微孢子虫及其在蝗害治理中的作用. 生物学通报**, 1999, **34** (2): 11～12.
[32] **张龙, 严毓骅, 石旺棚, 等. 协调应用蝗虫微孢子虫与卡死克防治东亚飞蝗. 中国生物防治**, 1999, **15** (2): 57～59.
[33] **郝树广, 秦启联, 王正军, 等. 国际蝗虫灾害的防治策略和技术: 现状与展望. 昆虫学报**, 2002, **45** (4): 531～537.