The susceptibilities to three organophosphate (OP) insecticides (malathion, chlorpyrifos, and phoxim), responses to three metabolic synergists (triphenyl phosphate (TPP), piperonyl butoxide (PBO), and diethyl maleate (DEM)), activities of major detoxification enzymes (general esterases (ESTs), glutathione S-transferases (GSTs), and cytochrome P450 monoxygenases (P450s)), and sensitivity of the target enzyme acetylcholinesterase (AChE) were compared between a laboratory-susceptible strain (LS) and a field-resistant population (FR) of the oriental migratory locust, Locusta migratoria manilensis (Meyen). The FR was significantly resistant to malathion (57.5-fold), but marginally resistant to chlorpyrifos (5.4) and phoxim (2.9). The malathion resistance of the FR was significantly diminished by TPP (synergism ratio: 16.2) and DEM (3.3), but was unchanged by PBO. In contrast, none of these synergists significantly affected the toxicity of malathion in the LS. Biochemical studies indicated that EST and GST activities in the...
FR were 2.1- to 3.2-fold and 1.2- to 2.0-fold, respectively, higher than those in the LS, but there was no significant difference in P450 activity between the LS and FR. Furthermore, AChE from the FR showed 4.0-fold higher activity but was 3.2-, 2.2-, and 1.1-fold less sensitive to inhibition by malaoxon, chlorpyrifos-oxon, and phoxim, respectively, than that from the LS. All these results clearly indicated that the observed malathion resistance in the FR was conferred by multiple mechanisms, including increased detoxification by ESTs and GSTs, and increased activity and reduced sensitivity of AChE to OP inhibition. Arch Insect Biochem Physiol © 2008 Wiley Periodicals, Inc.

Keywords: Locusta migratoria manilensis; organophosphate resistance; resistance mechanism; detoxification enzyme; acetylcholinesterase

INTRODUCTION

The oriental migratory locust, Locusta migratoria manilensis (Meyen) (Orthoptera: Acrididae), is one of the most important insect pests of prairies and croplands. In recent years, the destructive outbreaks of locust had been increasing in China due to the unwonted climate and the deteriorated ecological environment (Chen, 2000; Xia, 2002). Insecticide resistance was also an important factor contributing to the outbreaks (Yang, 2002).

In the past, control of L. migratoria manilensis mainly relied on organochlorine insecticides (Zhang et al., 2006; Zhu, 1999). Since the mid-1980s, organochlorines were banned mainly due to their chronic toxicity and environmental impact, and organophosphate (OP) insecticides were introduced to control the locust. Malathion, a major OP insecticide, has been used to control the locust for more than 20 years in China due to its rapid degradation in the environment (Zhu, 1999). In Huanghua County, Hebei Province of China, however, growers have complained of control failures by using malathion. He et al. (2004) reported that the locust from Huanghua County has developed OP resistance.

Two major resistance mechanisms, including increased metabolism [elevated cytochrome P450 monooxygenases (P450s), general esterases (ESTs), and glutathione S-transferases (GSTs) activities] and target site insensitivity [altered acetylcholinesterase (AChE) and altered sodium channel (kdr-type) resistance] have been detected in numerous pests (Rufingier et al., 1999; Zhu and He, 2000; Hemingway, 2000; Chen et al., 2007). AChE, as the target of organophosphates and carbamates, displays a lower affinity to some of these insecticides in many resistant insects (Delorme et al., 1997; Huang and Han, 2007). The change in affinity of AChE is proven to be the result of one or several point mutations as observed in cotton aphid (Li and Han, 2004). OPs and carbamates can be potentially metabolized by ESTs, GSTs, and P450s, and increased activity of any of these enzymes may cause resistance. Qu et al. (2003) reported that ESTs and P450s all played important roles in detoxification of OPs in Chilo suppressalis. Lee et al. (2000) demonstrated multiple resistance mechanisms, including altered AChE, elevated EST and P450 activities, were responsible for resistance in four resistant strains of Blattella germanica from Malaysia.

Preliminary studies suggested that reduced OP susceptibility in locust populations might attribute to increased EST activity and decreased sensitivity of AChE (He et al.,
2004; Ma et al., 2004). However, resistance levels of locust populations to different insecticides are still not known due to the absence of a “true” insecticide-susceptible strain of the locust. In addition, very little has been known about the mechanism of insecticide resistance in the locust populations.

The objectives of this study were to (1) evaluate the resistance spectrum of a field locust population using three selected OP insecticides; (2) examine the level of synergism in vivo with triphenyl phosphate (TPP), piperonyl butoxide (PBO), and diethyl meleate (DEM) to gain knowledge on possible metabolic resistance mechanisms; (3) investigate major biochemical mechanisms (i.e., enhanced levels of ESTs, GSTs, and P450s and AChE sensitivity) of resistance to OPs. Elucidation of the resistance spectrum and resistance mechanisms in *L. migratoria manilensis* will help us to make knowledge-based selections of insecticides for locust control and resistance management in the field.

**MATERIALS AND METHODS**

**Insects**

The susceptible laboratory strain (LS) of *L. migratoria manilensis* was provided by the Insect Protein Co., Ltd. of Cangzhou City in China. The locusts were originally collected from the field in 1997 and had been reared indoors on an artificial diet without exposure to any insecticides for 10 years. The field-resistant population (FR) of the locust was collected from Huanghua County, Hebei Province in China in 2007. The habitat of the FR is seacoast breeding area where insecticides have been used frequently in recent years (Ma et al., 2004).

**Chemicals**

Bicinchoninic acid solution (BCA), acetylthiocholine iodide (ATC), 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), malaoxon (O,O-dimethyl-S-1,2-di(carboethoxy)ethyl phosphorothiolate), chlorpyrifos (O,O-Diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate, purity 99.5%), fast blue B salt (O-dianisidine, tetrazotized), α-naphthol, β-naphthol, α-naphthyl acetate (α-NA), α-naphthyl butyrate (α-NB), β-naphthyl acetate (β-NA), piperonyl butoxide (PBO), β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt (NADPH), phenylmethyl sulfonyl fluoride (PMSF), and propylthiouracil (PTU), 1, 4-dithiothreitol (DTT), 1, 2-dechloro-4-nitrobenzene (DCNB), and p-nitrobenzene chloride (pNBC) were purchased from Sigma Chemical Co. (St. Louis, MO). Malathion (O, O-dimethyl-S-1, 2-di(carboethoxy)ethyl phosphorodithioate, purity 99.5%) and chlorpyrifos oxon (O, O-diethyl O-(3, 5, 6-trichlorl-2-pyridinyl) phosphate, purity 99.5%) were purchased from Chem Service (West Chester, PA). Bovine serum albumin (BSA) was purchased from Bio-Rad Laboratories (Hercules, CA). Reduced glutathione (GSH) and ethylenediamine tetraacetic acid (EDTA) were purchased from Bio Basic Inc (Shanghai, China). Triton X-100 and 1-chloro-2, 4-dinitrobenzene (CDNB) were purchased from Shanghai Sangon Biological Engineering Technology Co., Ltd., in China. Triphenyl phosphate (TPP) was from Beijing Chemical Reagent Co., Ltd., and diethyl meleate (DEM) was from Shanghai Chemical Reagent Co., Ltd., in China. Phoxim (benzoyl cyanide-O-(diethoxyphosphinothioyl)oxime, 98%) was obtained from ShenYang Research Institute of Chemical Industry in China.
Toxicity Bioassay

The susceptibilities of the locust to three OPs were evaluated using topical application. Insecticides were diluted to six different concentrations with acetone. Each of 19–22 fifth-instar nymphs in one replicate was topically applied with 5 μl of each insecticide solution or acetone (control) in the abdomen between the second and third sterna. For synergism assays, the synergist (TPP, PBO, or DEM) was diluted in acetone and the locusts were pre-treated with the 50-μg/locust for 1 h. Then the synergist-pretreated locusts were topically applied with insecticide of six different concentrations. Mortality was assessed after the treated locusts were maintained at room temperature (ca. 24°C) for 24 h. Mortality data from the insecticides bioassay and synergism assays were analyzed for the LD₅₀ values and their 95% confidence intervals (95% CIs) by probit analysis using the SPSS program (SPSS Inc., Chicago, IL). Resistance ratios (RR) were estimated at the LD₅₀ level as RR = LD₅₀ of FR/LD₅₀ of the LS. The synergism ratios (SR) were calculated by the formula of LD₅₀ value of insecticide alone/LD₅₀ value of insecticide after synergist pre-exposures.

Enzyme Preparation

The enzymes were prepared by homogenizing each of 24 locust thoraces for analyses of EST, GST, and P450 activities or each of 24 locust heads for analysis of AChE in 1 ml of an appropriate buffer on an ice-bath. 0.1 M sodium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM DTT, 1 mM PTU, and 1 mM PMSF was used for P450 preparation whereas 0.1 M sodium phosphate buffer (pH 7.5) containing 0.3% Triton X-100 was used for GST, EST and AChE assays. The homogenates were centrifuged at 15,000 g for 20 min at 4°C, and the supernatants collected as enzyme resources. Enzyme preparations of the 24 heads were divided into four groups. The enzyme preparations within each group, which represented 6 locusts, were combined as a replicate. The enzyme preparations were used to compare AChE kinetics and sensitivity to each of three selected OP compounds between the FR population and LS strain.

Protein Assay

Protein contents of enzyme preparations were determined according to Smith et al. (1985) using bovine serum albumin (BSA) as a standard. Measurements were performed with the SpectraMax 190 microplate reader and SOFTmax computer software (Molecular Devices, Menlo Park, CA) at 560 nm (Zhu and Clark, 1994).

Detoxification Enzyme Assay

The P450 activities were determined according to the method reported by Hansen and Hodgson (1971). One hundred microliters of 2 mM p-nitroanisole solution and 90 μl enzyme stock solution were added to each well of a microplate and mixed. After incubation for 2 min at 27°C, the reaction was initiated by adding 10 μl of 9.6 mM NADPH. Then, the optical density at 405 nm was immediately recorded at intervals of 25 s for 10 min using the microplate reader.

GST activities were assayed based on the method of Yang et al. (2002) with some modifications using CDNB, DCNB, and pNBC as substrates. Briefly, 10 μl of enzyme preparation was mixed with 190 μl of CDNB-GSH solution (10.35 mM GSH: 200 mM CDNB = 188:2 (v/v)) for CDNB activity analysis. For DCNB and pNBC, the reaction...
solution contained 20 µl of enzyme solution, 180 µl of DCNB/pNBC-GSH solution (10.35 mM GSH: 200 mM DCNB/pNBC = 178:2 (v/v)). The absorbance for GST activity was immediately read for 1 min at 340 nm for CDNB, 344 nm for DCNB, and 310 nm for pNBC using the microplate reader. All assays were corrected for non-enzymatic conjugation using the same mixtures except for using 10 µl of 0.1 M phosphate buffer (pH 7.5) containing 0.3% (v/v) TritonX-100 instead of the enzyme preparation. GST activities were calculated using the extinction coefficients of 9.6 mM$^{-1}$ cm$^{-1}$ for CDNB, 10.0 mM$^{-1}$ cm$^{-1}$ for DCNB, and 1.9 mM$^{-1}$ cm$^{-1}$ for pNBC.

The EST activities were assayed by the method of Zhu and He (2000) using α-NA, α-NB, and β-NA as substrates. Absorbance was determined at 600 nm for α-NA and α-NB, 560 nm for β-NA with the microplate reader.

**Assays of Activity, Kinetics, and Sensitivity of AChE**

AChE activities were determined based on the method of Zhu and Clark (1994) using ATC as a substrate. The AChE activity was determined using the microplate reader. Kinetic parameters of AChE were determined based on the AChE activities at 12 substrate concentrations ranging from 3.9 µM to 8 mM as previously described in the assay of AChE activity. The enzyme kinetic parameters, Michaelis–Menten constant ($K_m$) and maximal velocity ($V_{max}$), were estimated using Hanes transformations (Henderson, 1992). Sensitivity analysis of AChE to three OP compounds (malaoxon, chlorpyrifos oxon, and phoxim) was performed as previously described by Zhu and Clark (1995). The bimolecular rate constant ($k_i$) was determined according to the method of Aldridge and Davison (1952).

**Non-Denaturing Polyacrylamide Gel Electrophoretic Analysis of ESTs and AChE**

Non-denaturing polyacrylamide gel electrophoresis (PAGE) of EST and AChE was carried out with a DYCZ-24D Electrophoresis Cell (Liu-Yi, Beijing, China). Each lane was loaded with the same amount of sample. The PAGE of ESTs was performed as previously described (He et al., 2004) and gels were stained for EST activity using α-NA or β-NA as substrates and fast garnet GBC as the chromogenic agent (Manchenko, 1994; Zhu and He, 2000). For PAGE analysis of AChE, the gel (4 and 7% acrylamide in stacking and separating gels, respectively) was run at a constant voltage of 150 V on an ice-bath. The AChE bands were visualized by incubating the gels in staining mixture overnight at room temperature (Karnovsky and Roots, 1964; Zhu and Brindley, 1992).

**RESULTS**

**Resistance Spectrum to Selected OP Insecticides**

The susceptibilities of the FR and LS of *L. migratoria manilensis* to three OP insecticides are presented in Table 1. Compared with the LS, the FR collected from Huanghua County showed low to significant levels of resistance to all the insecticides examined. The resistance levels were 2.9-fold to phoxim, 5.4-fold to chlorpyrifos, and 57.5-fold to malathion.

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Table 2 shows the synergism of TPP, PBO, and DEM on malathion in the FR and LS of the locust. The malathion resistance of the FR was significantly diminished by TPP (synergism ratio: 16.2) and DEM (3.3), but was unchanged by PBO (0.9). In contrast, none of these synergists significantly affected the toxicity of malathion in the LS. These results suggested that both ESTs and GSTs were involved in OP resistance in the FR, whereas P450s were unlikely to be involved in the resistance simply because PBO did not synergize the toxicity of malathion.

Activities of Detoxification Enzymes

To further examine whether or not these common detoxification enzymes were involved in OP resistance in the FR, we compared the EST, GST, and P450 activities of the fifth-instar nymphs between the FR and LS of the locust (Table 3). The results showed that the mean of the EST activity in the FR was 3.2-, 2.1-, and 2.9-fold higher than that of the LS when α-NA, α-NB, and β-NA were used as substrates, respectively. The non-denaturing PAGE analysis of ESTs for both the FR and LS displayed at least 11 different bands of EST activity when α-NA and β-NA were used as substrates (Fig. 1). However, the bands indicating EST activity in the FR were much more intense than those in the LS, particularly for those identified as ESTs 2, 3, 4, 5, and 6.

The GST activity of the FR and LS was compared using three substrates: CDNB, DCNB, and pNBC. The GST activity was 2.0- and 1.9-fold higher in the FR than in the LS when CDNB and pNBC were used as substrates, respectively. However, no difference was observed between the FR and LS when DCNB was used as a substrate (Table 3). For P450 activity assay, there was no significant difference between the FR and LS of the locust (Table 3).

Characterization of AChE

The specific activity of AChE differed significantly between the FR and LS of the locust (Table 4). The specific activity of AChE from the FR was about 4.0-fold higher than that of the LS. Significant increase of AChE activity in the FR was also confirmed by non-denaturing PAGE, showing remarkably increased intensity in the FR when gel was stained for AChE activity (Fig 2).
The affinities and hydrolyzing efficiencies of AChE from the FR and LS were determined based on kinetic analysis. The affinity of AChE to the substrate ATC from the FR was 2.6-fold lower than that from the LS as indicated by their $K_m$ values (Table 4). In contrast, the hydrolyzing efficiency of AChE in the FR was 2.3-fold higher than that in the LS based on their $V_{\text{max}}$ values.

The sensitivity of AChE was compared between the FR and LS using three OPs (malaoxon, chlorpyrifos oxon, and phoxim) (Table 5). Our results indicated that AChE from the FR was 3.2- and 2.2-fold less sensitive ($P<0.05$) than that from the LS to malaoxon and chlorpyrifos oxon, respectively; however, no significant difference was

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**Table 2. Changes of the Susceptibility in the Fifth-Instar Nymphs of a Laboratory Susceptible Strain (LS) and a Field Resistant Population (FR) of L. migratoria manilensis to Malathion after Pre-exposed to PBO, TPP and DEM**

<table>
<thead>
<tr>
<th>Locust</th>
<th>Treatment</th>
<th>$n^a$</th>
<th>$X^2$</th>
<th>$P^*$</th>
<th>Slope ± SE</th>
<th>$LD_{50}$ (μg/g body weight) (95% CL)</th>
<th>SR$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>Malathion</td>
<td>376</td>
<td>1.52</td>
<td>0.72</td>
<td>1.67 ± 0.19</td>
<td>2.36 (1.54–3.68)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>+ TPP</td>
<td>367</td>
<td>2.51</td>
<td>0.65</td>
<td>1.50 ± 0.15</td>
<td>1.74 (1.11–2.75)</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>+ PBO</td>
<td>378</td>
<td>1.19</td>
<td>0.88</td>
<td>1.56 ± 0.23</td>
<td>2.10 (1.35–3.35)</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>+ DEM</td>
<td>366</td>
<td>4.38</td>
<td>0.42</td>
<td>1.19 ± 0.12</td>
<td>2.05 (1.22–3.60)</td>
<td>1.2</td>
</tr>
<tr>
<td>FR</td>
<td>Malathion</td>
<td>378</td>
<td>0.88</td>
<td>0.68</td>
<td>1.71 ± 0.99</td>
<td>135.65 (68.58–221.63)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>+ TPP</td>
<td>338</td>
<td>2.38</td>
<td>0.67</td>
<td>1.87 ± 0.12</td>
<td>8.37 (5.00–13.17)</td>
<td>16.2$^c$</td>
</tr>
<tr>
<td></td>
<td>+ PBO</td>
<td>340</td>
<td>1.72</td>
<td>0.75</td>
<td>0.75 ± 0.06</td>
<td>143.41 (70.22–237.94)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>+ DEM</td>
<td>337</td>
<td>1.62</td>
<td>0.80</td>
<td>1.16 ± 0.29</td>
<td>40.95 (21.73–98.81)</td>
<td>3.3$^c$</td>
</tr>
</tbody>
</table>

* $P \geq 0.05$ indicates a significant fit between the observed and expected regression lines in a probit analysis.

$^a$ Number of the locust nymphs tested in each bioassay.

$^b$ SR: synergism rate that was calculated by the $LD_{50}$ value of insecticide alone/$LD_{50}$ value of insecticide after synergist pretreatment.

$^c$ There is significant synergism based on non-overlap of the 95% CLs of the $LD_{50}$ values between malathion only and malathion after synergist pretreatment.

**Table 3. Comparisons of Specific Activities of Detoxification Enzymes in a Laboratory Susceptible Strain (LS) and a Field Resistant Population (FR) of L. migratoria manilensis**

<table>
<thead>
<tr>
<th>Enzyme/substrate</th>
<th>Locust</th>
<th>Enzyme activity$^a$</th>
<th>Ratio of enzyme activity (FR/LS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESTs/α-NA</td>
<td>LS</td>
<td>0.22 ± 0.03</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>0.71 ± 0.08*</td>
<td>3.2</td>
</tr>
<tr>
<td>ESTs/α-NB</td>
<td>LS</td>
<td>0.27 ± 0.07</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>0.57 ± 0.06*</td>
<td>2.1</td>
</tr>
<tr>
<td>ESTs/α-NA</td>
<td>LS</td>
<td>0.24 ± 0.03</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>0.69 ± 0.14*</td>
<td>2.9</td>
</tr>
<tr>
<td>GSTs/CDNB</td>
<td>LS</td>
<td>184.68 ± 27.33</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>372.76 ± 141.19*</td>
<td>2.0</td>
</tr>
<tr>
<td>GSTs/DCNB</td>
<td>LS</td>
<td>11.21 ± 2.22</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>13.06 ± 3.06</td>
<td>1.2</td>
</tr>
<tr>
<td>GSTs/pNBC</td>
<td>LS</td>
<td>6.28 ± 0.70</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>12.11 ± 3.61*</td>
<td>1.9</td>
</tr>
<tr>
<td>P450s/p-NA</td>
<td>LS</td>
<td>10.87 ± 0.91</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>9.52 ± 1.46</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Indicates that the mean from the FR is significantly different from that of the LS ($P>0.05$) by Student’s $t$-test.

$^a$ Results are the means ± S.D. of four replicates (total 24 insects) for every strain, each replicate with triplicate analyses. The units of EST and GST activities are μmol/min/mg protein, and the unit of P450 activity is mOD/min/mg protein.

The affinities and hydrolyzing efficiencies of AChE from the FR and LS were determined based on kinetic analysis. The affinity of AChE to the substrate ATC from the FR was 2.6-fold lower than that from the LS as indicated by their $K_m$ values (Table 4). In contrast, the hydrolyzing efficiency of AChE in the FR was 2.3-fold higher than that in the LS based on their $V_{\text{max}}$ values..

The sensitivity of AChE was compared between the FR and LS using three OPs (malaoxon, chlorpyrifos oxon, and phoxim) (Table 5). Our results indicated that AChE from the FR was 3.2- and 2.2-fold less sensitive ($P<0.05$) than that from the LS to malaoxon and chlorpyrifos oxon, respectively; however, no significant difference was
detected in the sensitivity of AChE to phoxim based on the $k_i$ values between the FR and LS.

**DISCUSSION**

*L. migratoria manilensis* is a serious pest and has been mainly controlled by application of OP insecticides in China. Thus, it is not surprising that many field populations have developed resistances to OP insecticides. In the current study, a field population (i.e., FR) of the locust collected in Huanghua County, Hebei province of China, was proved to have marginal to high resistance to selected OP insecticides compared with the

**Table 4. Activities and kinetic parameters of AChE prepared from a laboratory susceptible strain (LS) and a field resistant population (FR) L. migratoria manilensis in hydrolyzing the substrate acetylthiocholine (ATC)**

<table>
<thead>
<tr>
<th>Locust</th>
<th>AChE Activity (μmol/min/mg)</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (μmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>3.62 ± 1.73</td>
<td>28.37 ± 5.83</td>
<td>7.40 ± 1.92</td>
</tr>
<tr>
<td>FR</td>
<td>14.63 ± 5.79</td>
<td>74.05 ± 10.14</td>
<td>16.95 ± 7.39</td>
</tr>
</tbody>
</table>

*Indicated that the mean from the FR is significantly different from that of the LS ($P > 0.05$) by Student’s $t$-test. Results are the mean ± SD of 24 individual heads ($n = 24$), and results on AChE kinetics are the mean ± SD of four replicates ($n = 4$); each with triplicate analyses.

Figure 1. Comparisons of esterase (EST) profiles between a laboratory-susceptible strain (LS) and a field-resistant population (FR) of *L. migratoria manilensis* by using non-denaturing PAGE. Each lane was loaded with 80 mg of total protein from each sample. Four lanes represented 4 biological replicates for LS and FR, respectively. Gels (10% separating gel and 4% stacking gel) were run at a constant voltage of 200 V for 50 min, and the ESTs were stained for their activity using α- and β-naphthyl acetate as substrates.
For malathion, the resistant level for the FR (LD50: 135.65 mg/g body weight) was approximately 9-fold higher than that determined in 2002 (He et al., 2004), which implied that the field population was continually controlled with malathion.

Numerous studies have demonstrated that the increased metabolic detoxifications were involved in OP resistance in many insect pests. In the Japanese encephalitis vector mosquitoes, *Culex tritaeniorhynchus*, increased GST and EST activities were found to play an important role in resistance to malathion and propoxur. The role of the laboratory-susceptible strain (i.e., LS) (Table 1). For malathion, the resistant level for the FR (LD50: 135.65 mg/g body weight) was approximately 9-fold higher than that determined in 2002 (He et al., 2004), which implied that the field population was continually controlled with malathion.

Table 5. Biomolecular rate constants ($k_i$) of three organophosphate compounds in the inhibition of AChE from a laboratory susceptible strain (LS) and a field resistant population (FR) of *L. migratoria manilensis*

<table>
<thead>
<tr>
<th>Organophosphate</th>
<th>Locust</th>
<th>$k_i \pm SD \ (M^{-1} \ min^{-1})$</th>
<th>Ratio of $k_i$ (LS/FR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaoxon</td>
<td>LS</td>
<td>$(9.88 \pm 1.14) \times 10^6$</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>$(3.07 \pm 0.86) \times 10^6$</td>
<td>—</td>
</tr>
<tr>
<td>Chlorpyrifos-oxon</td>
<td>LS</td>
<td>$(9.78 \pm 0.35) \times 10^6$</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>$(4.55 \pm 0.49) \times 10^8$</td>
<td>—</td>
</tr>
<tr>
<td>Phoxim</td>
<td>LS</td>
<td>$(1.04 \pm 0.12) \times 10^5$</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>$(9.15 \pm 0.23) \times 10^4$</td>
<td>—</td>
</tr>
</tbody>
</table>

*Indicates that the mean from the FR is significantly different from that of the LS ($P < 0.05$) by Student’s *t*-test. *Results are the means $\pm$ S.D. of four determinations ($n = 4$).

laboratory-susceptible strain (i.e., LS) (Table 1). For malathion, the resistant level for the FR (LD50: 135.65 mg/g body weight) was approximately 9-fold higher than that determined in 2002 (He et al., 2004), which implied that the field population was continually controlled with malathion.

Numerous studies have demonstrated that the increased metabolic detoxifications were involved in OP resistance in many insect pests. In the Japanese encephalitis vector mosquitoes, *Culex tritaeniorhynchus*, increased GST and EST activities were found to play an important role in resistance to malathion and propoxur. The role of the
ESTs in OP resistance appeared to sequestrate insecticides (Karunaratne and Hemingway, 2000). In *Culex quinquefasciatus*, increased EST activity was also found to be a major resistance mechanism against malathion (Selvi et al., 2007).

Synergists, which block certain insecticide metabolic enzymes, can be used to enhance the toxicities of insecticides against insect pests. When an insecticide bioassay is conducted both in the presence and absence of a relatively specific synergist, the degree of synergism can suggest whether or not certain detoxification enzymes are involved in metabolizing the pesticides (Yang et al., 2001). In this study, we used three synergists (TPP, an EST inhibitor; PBO, a P450 inhibitor; and DEM, a GST inhibitor) to evaluate whether these synergists could enhance the toxicity of malathion. The significant synergistic effect of TPP and DEM on the toxicity of malathion in the FR indicated that both ESTs and GSTs played important roles in detoxification of malathion in the FR (Table 2).

To confirm possible involvements of these major detoxification enzymes in malathion resistance in the FR, we compared the EST, GST, and P450 activities between the FR and LS. Our results indicated that EST and GST activities, except for P450 activity, in the FR were significantly higher than those in the LS. In addition, the non-denaturing PAGE analysis of ESTs in the FR showed similar banding patterns but increased staining intensity of the α-NA- and β-NA-hydrolyzing ESTs compared with the LS, which further verified the significant difference in EST activities between the FR and LS. Indeed, these results were consistent with those of our synergism bioassays showing significant synergistic effects of TPP and DEM on the toxicity of malathion in the FR. Therefore, ESTs and GSTs played major roles in conferring malathion resistance in the FR, whereas P450s were less likely to be important in detoxification of malathion in the FR.

Increased AChE activity may confer OP and/or carbamate resistance in insects even though the enzyme is sufficiently sensitive to these insecticides (Gao and Zhu, 2002). Increased AChE activity associated with OP-resistance has been documented in *Drosophila melanogaster* (Berrada and Fournier, 1997), *Aonidiella aurantii* (Levitin and Cohen, 1998), and *Schizaphis graminum* (Gao and Zhu, 2002). Our study revealed that the specific activity of AChE towards ATC was 4.0-fold higher in the FR than in the LS, and the PAGE analysis of AChE activity showed the same trend. These results indicated that the increased AChE activity in the FR appeared to be associated with OP resistance.

Decreased sensitivity of AChE has also been recognized as one of the common mechanisms of resistance to OP compounds in many insects (Qu et al., 2003; Huang et al., 2007). Our kinetic analyses clearly showed that AChE from the FR was significantly different from that of the LS. Such differences were reflected by not only the reduced sensitivity levels to different OP inhibitors (Table 5), but also the decreased affinity to ATC (Table 4). Furthermore, there was a strong correlation between resistance and AChE sensitivity levels with the three pairs of OP compounds examined in this study (Fig. 3). Specifically, the resistance levels of 57.5-, 5.4-, and 2.9-fold to malathion, chlorpyrifos, and phoxim were associated with the reduced AChE sensitivity levels of 3.2-, 2.2-, and 1.1-fold to inhibition by malaoxon, chlorpyrifos oxon, and phoxim, respectively. These results strongly suggested that reduced sensitivity of AChE also played a major role in contributing to OP resistance in the FR. Similar results have also been reported concerning malathion resistance in many other insect species including *Blastiella germanica* (Bull et al., 1989) and *Culex quinquefasciatus* (Bisset et al., 2000).
In conclusion, malathion resistance in the FR was partly due to the EST- and GST-mediated metabolic detoxification as evidenced by significant synergistic effect of DEF and TPP on the toxicity of malathion, and significantly increased GST and EST activities. In addition, increased activity and reduced sensitivity of AChE also contributed to malathion resistance in the FR. These findings are expected to help researchers detect and monitor insecticide resistance in *L. migratoria manilensis*, and to devise effective chemical control methods for this important insect pest in the field.

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**LITERATURE CITED**


