Bulletin of Entomological Research (2009) **99**, 611–617
doi:10.1017/S0007485309006725

First published online 5 May 2009

Multiple mechanisms responsible for differential susceptibilities of *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) to pirimicarb

Y. Lu and X. Gao*

Department of Entomology, China Agricultural University, Beijing 100193, China

Abstract

Both *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) are the most important pests of wheat in China and usually coexist on the late period of wheat growth. Pirimicarb was introduced into China for wheat aphid control in early 1990s, and differential susceptibilities of *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) to pirimicarb have been observed. A bioassay exhibited that *Rhopalosiphum padi* possessed significantly higher susceptibility to pirimicarb than *Sitobion avenae*. The addition of synergists DEF, an esterase inhibitor, PBO, a cytochrome P450 monooxygenase inhibitor, and DEM, a glutathione *S*-transferase inhibitor, resulted in apparent reductions in the differential susceptibilities, suggesting the involvement of the above three detoxification enzymes in the differential susceptibility to pirimicarb between *Sitobion avenae* and *Rhopalosiphum padi*. A biochemical analysis showed that the activities of carboxylesterases and glutathione *S*-transferases were significantly higher in *Sitobion avenae* than in *Rhopalosiphum padi*, consistent with the results of synergism. Acetylcholinesterase is the target enzyme of pirimicarb and the sensitivity of acetylcholinesterase to pirimicarb was significantly higher in *Rhopalosiphum padi* than in *Sitobion avenae*. The combined results suggest that multiple mechanisms are likely to be responsible for differential susceptibilities to pirimicarb between *Sitobion avenae* and *Rhopalosiphum padi*. The results obtained from this study should be helpful in the rational applications of insecticides.

Keywords: Differential susceptibility, *Sitobion avenae*, *Rhopalosiphum padi*, acetylcholinesterase, carboxylesterase, glutathione *S*-transferase

(Accepted 3 November 2008)

Introduction

Both *Sitobion avenae* (Fabricius) (Sa) and *Rhopalosiphum padi* (Linnaeus) (Rp) are the most important pests, causing damage by direct feeding and as vectors of numerous plant pathogenic viruses and usually coexist on the late period of wheat growth in China. The control of Sa and Rp is primarily dependent on the application of insecticides, but the resistance of both aphid species developed slowly in virtue of their short outbreak periods (Chen *et al*., 2007). According to the Chinese cropping system, many natural enemies live through the winter in wheat fields and migrate to cotton or corn fields after the wheat harvest. Therefore, selective insecticides are recommended to conserve natural enemies (Gao *et al*., 1991; Chen *et al*., 2007). Pirimicarb is a selective

*Author for correspondence
Fax: +86-1-62732974
E-mail: gaoxiwu@263.net.cn

carbamate insecticide and is often applied in aphid management programs (Gao *et al*., 1991). Pirimicarb has been used for the control of wheat aphids since the 1990s in China. Difference in the efficacy of pirimicarb between the above two aphid species has been found in wheat field of He'nan (Liu *et al*., 2001). Bioassays showed that both aphid species differ in their susceptibility to insecticides. Rp was more susceptible than Sa to most of insecticides used for wheat aphid control.

The objectives of this research were: (i) to evaluate the synergistic effects of PBO, DEF and DEM on differential susceptibilities to pirimicarb; (ii) to determine the activity difference in general esterases and glutathione *S*-transferases (GST); and (iii) to determine the sensitive difference of acetylcholinesterase (AChE) and carboxylesterase (CarE) to pirimicarb between the two aphid species. These research results are very significant to elucidate differential susceptibility mechanisms of pirimicarb between the two aphid species and to develop an efficient resistance management strategy.

Materials and methods

Insects

Colonies of both aphid species were established from field collections in May 2005 from the same wheat field of the Agricultural Experiment Station, China Agricultural University. They have been maintained in the laboratory without insecticide exposure since May 2005. Two colonies were maintained on wheat seedlings at 18–25°C, a photoperiod of 17:7 h (L : D) and relative humidity 50–70% (Lu & Gao, 2007). Apterous adults were collected in 1.5 ml microcentrifuge tubes and immediately stored at −80°C.

Chemicals

Pirimicarb (95% a.i.) was obtained from Wuxi Ruize Chemical Co. Ltd, China. Piperonyl butoxide (PBO, 98%) and S,S,S-tributylphosphorotrithioate (DEF, 98%) were purchased from Chem Service (West Chester, PA). Acetylthiocholine iodide (ATCh), butyrylthiocholineiodide (BuTCh), acetyl-(β-methyl) thiocholine iodide (MeTCh), propionylthiocholine iodide (PrTCh), bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), eserine sulfate, sodium dodecyl sulfate (SDS), α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), α-naphthyl caprylate (α-NC), α-naphthyl butyrate (α-NB), ethylenediaminetetraacetic acid (EDTA), fast blue RR salt, 1,2-dichloro-4-nitrobenzene (DCNB), diethyl maleate (DEM), TritionX-100 and 1,5-bis(4-allyldimethyl-ammoniumphenyl)-pentan-3-one dibromide (BW284C51) were purchased from Sigma Chemical Company (St Louis, USA). Coomassie brilliant blue G-250 was purchased from Fluca (Buchs, Switzerland). 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and fast blue B salt (*O*-dianisidine, tetrazotized) were purchased from Fluka Chemical Company (St Quentin, France).

Insecticide bioassays

Insecticide toxicity was assayed using the method of residual film in glass tubes described by Shotkoski *et al*. (1990) and Shufran *et al*. (1997) with some modifications to the glass tubes (diameter: 2 cm; inner surface: 36 cm^2). According to the method of residual film in glass tubes, each insecticide was diluted to the 6–7 required concentration in analytical grade acetone. An aliquot of 200 µl insecticide-acetone solution was applied to every tube and was immediately rotated by using a micro-rotator (American Wheaton Company). Twenty aphids were treated for each concentration with three replications. Controls were used in tubes treated with acetone only. The treated aphids were reared routinely and mortality was checked after three hours, according to the method of Shotkoski *et al*. (1990) and Shufran *et al*. (1997), with some modifications. Adults failing in exhibiting repetitive (i.e. non-reflex) movement of more than one leg (after gentle prodding, if necessary) were assumed dead (Moores *et al*., 1996).

Synergism bioassays

Toxicity of pirimicarb in the presence and absence of synergists, DEF, PBO and DEM, was evaluated by the bioassay method, as described previously. The maximum sublethal dose and treatment time for synergist were determined according to Liu & Yue (2000), Scott *et al*. (2000), Yang *et al*. (2001) and Mohammadi *et al*. (2007). Synergists, DEF, PBO and DEM, were applied at the maximum sublethal dose (final concentrations were 0.039 µg cm^{-2} for DEF, 0.028 µg cm^{-2} for PBO and 0.14 µg cm^{-2} for DEM, respectively) one hour before the insecticide treatment. The synergist alone was used as control. Mortality was recorded after three hours. Synergistic ratios were calculated using the conventional approach of dividing LC_{50} without the synergist by LC_{50} with the synergist.

AChE activity and inhibition

Batches of approximate 0.1 g frozen apterous adults were manually homogenized in 1 ml of 0.1 M ice-cold phosphate buffer (pH7.5 containing 0.5% Triton X-100). The procedure was performed on ice. Homogenates were centrifuged at 4°C, 10,000 *g* (Eppendorf centrifuge 5417R, Germany) for 30 min. The supernatant was used as an enzyme source for measuring the activity of AChE and proteins. Acetylcholinesterase (AChE) activity was measured using the method of Ellman *et al*. (1961) with minor modifications by Gao (1987). Briefly, for each reaction, 25 µl substrate (5 mM) and 25 µl enzyme were incubated at 30°C for 15 min. The reaction was stopped by the addition of 900 µl DTNB (0.125 mM) with 40% ethanol, and the optical density (OD) was measured at 412 nm by spectrophotometer (Lambda Bio 40). The control samples contained no enzyme during the incubation. After the addition of the color reagent, appropriate amounts of enzyme solutions were added to the controls. ATCh, PrTCh, MeTCh and BuTCh were used as substrates for AChE activity measurements.

For the AChE sensitivity experiment, pirimicarb was first dissolved in acetone and then diluted into the desired concentrations with phosphate buffer (0.1 M, pH 7.5). An aliquot of 20 µl enzyme was incubated with 5 µl insecticide solution of different concentrations at 30°C for 5 min prior to the addition of the substrate, and the final concentration of acetone was less than 1%. Then, 25 µl ATCh (5 mM) were added to the mixture. The residual AChE activity was determined according to the above method. The value of median inhibition concentration (IC_{50}) for pirimicarb was determined based on log (inhibitor concentration) vs. probit (percentage of inhibition) linear regression.

https://doi.org/10.1017/S0007485309006725 Published online by Cambridge University Press

Purification of AChE from both aphid species was performed by affinity chromatography using procainamide as an affinity ligand (Gao & Zhu, 2001).

CarE activity and inhibition

To determine carboxylesterase (CarE) activities, batches of approximate 0.1 g frozen apterous adults were manually homogenized in 1 ml ice-cold, 0.04 M phosphate buffer (pH7.0). Homogenates were centrifuged at 4°C, 10,000 *g* (Eppendorf centrifuge 5417R, Germany) for 30 min, and the supernatants were collected as enzyme sources. CarE activities were measured at 30°C by the method of van Asperen (1962) with some modifications using α-NA, α-NB, α-NC and β-NA as substrates. For a separate sample, 1.8 ml homogenization buffer containing substrate (3×10^{-4} M) and eserine (3×10^{-4} M), and 50 µl enzyme diluted from the enzyme preparation with 0.04 M phosphate buffer (pH 7.0) were added to each reaction. The mixture was incubated for 15 min and the enzyme reaction was stopped by the addition of 900 µl fast blue B-SDS solution. The absorbance was determined at 600 nm for α-NA, α-NB and α-NC, and at 555 nm for β-NA using a spectrophotometer (Lambda Bio 40). The optical density (OD) values were converted to the production of naphthol $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein through naphthol standard curves and protein values.

The α-NA-hydrolyzing esterase activity in individual aphids (110 apterous adults for each species) was determined by the method of Moores *et al*. (1996), using the microplate assay format with some modifications. Briefly, a single aphid was homogenized in 60 µl ice-cold 0.04 M phosphate buffer (pH 7.0). The homogenates were centrifuged at 10,000 *g* (Eppendorf centrifuge 5417R, Germany) for 10 min at 4°C. A separate sample containing 50 µl supernatants, 150 µl substrate (α-NA 100 µM) and fast blue RR salt (1.5 mM) mixture filtered prior to use, and 50 µl buffer were added to each well of microplate, the total volume being 250 µl. Reactions were immediately monitored for 5 min with 10 s intervals at 450 nm, 25°C by a Thermomax microplate reader (Tecan Spectra). The activity of esterase was expressed as the slope of linear regressions.

For the sensitivity test, a stock solution of insecticide or synergist was prepared at 10 mM in acetone and diluted to the desired concentrations with phosphate buffer. Enzyme was incubated with the insecticide or synergist at 30°C for 15 min prior to the addition of the substrate, and the final concentration of acetone was less than 1%. A control was included for each experimental run. Each compound was used with at least five concentrations. The value of median inhibition concentration (IC_{50}) was determined based on log (inhibitor concentration) vs. probit (percentage of inhibition) linear regression.

Activity and kinetic analysis of GSTs

Glutathione *S*-transferase (GST) activity was determined in apterous adults of Sa and Rp using CDNB and DCNB as substrates as described by Habig *et al*. (1974). Batches of approximate 0.1 g frozen apterous adults were homogenized in 1 ml ice-cold, 0.1 M phosphate buffer (pH6.5) containing 1 mM EDTA. Homogenates were centrifuged at 10,000 *g* (Eppendorf centrifuge 5417R, Germany) for 30 min at 4°C, and the supernatants were collected as enzyme sources. For assay of GST activity, briefly, the assay mixtrue (the total volume was 900 µl) contained 1 mM CDNB or DCNB and 1 mM GSH. The assay was initiated by the addition of 50 µl enzyme for Rp (20 µl for Sa); the absorbance at 340 nm for CDNB or 345 nm for DCNB was monitored for 2 min with 10 s intervals by spectrophotometer (Lambda Bio 40). Controls without enzyme always accompanied each assay. Activity was calculated with an extinction coefficient of $9.6\ mM^{-1}cm^{-1}$ for CDNB or $8.5\ mM^{-1}cm^{-1}$ for DCNB. Enzyme activity was expressed as $nmol\ min^{-1}$ at 25°C, and the specific activity as $nmol \cdot min^{-1} \cdot mg^{-1}$ protein. Michalelis constants (K_m) and maximal velocities (V_{max}) were determined by double-reciprocal Lineweaver-Burk plots.

Determination of protein contents

Protein content of the enzyme preparations was determined according to the method of Bradford (1976) using BSA as standard.

Data analysis

Data of bioassays were analyzed using the SAS-probit program. All statistical tests were performed using a SAS computer program.

Results

Pirimicarb toxicity and synergism bioassay

Pirimicarb was 4.8-fold more toxic to Rp than to Sa. The LC_{50} values were $0.0052\ \mu g\ cm^{-2}$ for Rp and $0.0250\ \mu g\ cm^{-2}$ for Sa. DEF, an esterase inhibitor, PBO, a cytochrome P450 monooxygenase inhibitor, and DEM, a GST inhibitor, reduced the toxicity difference between both Rp and Sa, from 4.8-fold to 2.4-, 2.4- and 1.9-fold, respectively (table 1).

AChE characteristics

As the molecular target of carbamate and organophosphate insecticides, the characteristics of crude extract AChE from both Sa and Rp were investigated (table 2). Catalytic activities of AChE toward ATCh, PrTCh, MeTCh and BuTCh were 0.92, 0.70, 0.76 and 1.58 $nmol \cdot min^{-1} \cdot mg^{-1}$ protein in Sa, and 1.09, 0.69, 0.81 and 1.19 $nmol \cdot min^{-1} \cdot mg^{-1}$ protein in Rp, respectively. There was no significant difference in the catalytic activities of AChE crude extract toward ATCh, PrTCh and MeTCh between Sa and Rp, but the activity difference in BuTCh hydrolysis was observed between both aphid species. However, AChE activities after purification against ATCh, PrTCh, MeTCh and BuTCh were significantly higher in Sa than in Rp (table 3). The sensitivity of AChE from Sa was significantly different from Rp, the median inhibition concentration (IC_{50} value) on AChE crude extract was 5.37-fold higher for Sa than for Rp (table 4).

CarE activity and inhibition

The CarE activity was characterized spectrophotometerically against four surrogate ester substrates, α-NA, α-NB, α-NC and β-NA. The Sa exhibited significant higher CarE activities (3.23-, 1.69-, 2.79- and 1.34-fold for α-NA, α-NB, α-NC and β-NA, respectively) than Rp (table 5).

The frequency distribution of Rp with respect to the α-NA hydrolyzing esterase activity was significantly different

https://doi.org/10.1017/S0007485309006725 Published online by Cambridge University Press

Table 1. Comparison of pirimicarb toxicity, with or without synergists, between *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa).

Treatment	Insects	N[a]	Slope ± SE[b]	LC_{50}[c]	X^{2d}	TDR[e]
Pirimicarb	Rp	360	3.56 ± 0.34	0.0052(0.0046–0.0059)	1.35	1
	Sa	360	2.36 ± 0.23	0.0250(0.0211–0.0295)	4.13	4.81
Pirimicarb + DEF	Rp	360	2.43 ± 0.23	0.0023(0.0019–0.0027)	4.60	1
	Sa	360	2.83 ± 0.28	0.0056(0.0048–0.0065)	3.87	2.43
Pirimicarb + PBO	Rp	360	2.75 ± 0.41	0.0034(0.0022–0.0051)	6.40	1
	Sa	360	1.61 ± 0.20	0.0080(0.0064–0.0101)	2.15	2.35
Pirimicarb + DEM	Rp	360	2.10 ± 0.37	0.0032(0.0018–0.0056)	7.80	1
	Sa	360	2.10 ± 0.22	0.0062(0.0052–0.0074)	0.89	1.94

[a] Number of tested aphids.
[b] SE = standard error.
[c] Expressed in $\mu g\,cm^{-2}$; 95% CI of LC_{50} are given in parenthesis.
[d] Chi-square testing linearity of dose-mortality responses.
[e] TDR = the toxicity difference ratio based on LC_{50} values between Rp and Sa.

from the distribution of Sa. The esterase activity in Rp ranged from 20 to 100 mOD min^{-1} aphid, while from 60 to 200 mOD min^{-1} aphid in Sa (fig. 1).

The inhibition of pirimicarb and DEF on CarE activity was stronger in Sa than in Rp (table 6).

Kinetic analysis of GSTs

The specific activity of GSTs was significantly higher in Sa than in Rp when CDNB and DCNB were used as substrates (table 7). Kinetic analysis did not indicate statistically significant differences in the K_m values but showed a significant difference in the V_{max} values between Sa and Rp (table 8). These results indicated that the detoxification efficiency by GSTs was likely higher in Sa than in Rp.

Discussion

The toxicity difference of pirimicarb between the two aphid species was observed in wheat field (Liu *et al.*, 2001). This toxicity difference has affected the field efficacy of pirimicarb in wheat aphid control. After spraying with pirimicarb, the population of Sa was significantly more than that of Rp (unpublished data). Our bioassay results revealed a 4.8-fold difference in LC_{50} values between Sa and Rp, and the difference in LC_{50} values between both aphid species was statistically significant based on the criterion of failure of 95% confidence intervals to overlap. Therefore, the alternation or mixture of pirimicarb with other insecticides was considered in wheat aphid management programs because

Table 2. Comparison of specific activity of crude extract AChE isolated from *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa) using ATCh, PrTCh, MeTCh and BuTCh as substrates[a].

Substrate	Specific activity ± SE (nmol · min^{-1} · mg^{-1} protein)	
	Rp	Sa
ATCh	1.09 ± 0.45	0.92 ± 0.13
PrTCh	0.69 ± 0.12	0.70 ± 0.14
MeTCh	0.81 ± 0.15	0.76 ± 0.11
BuTCh	1.19 ± 0.43	1.58 ± 0.24*

[a] Data are the means of three determinations, means followed by * represent significant difference between Rp and Sa ($P < 0.05$).

Table 3. Comparison of specific activity of the purified AChE isolated from *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa) using ATCh, PrTCh, MeTCh and BuTCh as substrates[a].

Substrate	Specific activity ± SE (nmol · min^{-1} · mg^{-1} protein)	
	Rp	Sa
ATCh	20.10 ± 0.10	79.43 ± 2.40*
PrTCh	18.30 ± 0.46	72.03 ± 6.12*
MeTCh	16.43 ± 0.32	62.63 ± 5.32*
BuTCh	1.66 ± 0.06	5.50 ± 0.33b*

[a] Data are the mean of three determinations, mean followed by * represent significant difference between Rp and Sa ($P < 0.05$).

Table 4. Median inhibition concentration (IC_{50}) of pirimicarb to AChE from *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa)[a].

Inhibitor	IC_{50} ± SE ($\times 10^{-5}$ M)	
	Rp	Sa
Pirimicarb	1.01 ± 0.13	5.43 ± 0.69*

[a] Data are the mean of three determinations, mean followed by * represent significant difference between Rp and Sa ($P < 0.05$).

Table 5. Comparison of specific activity of CarE isolated from *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa) using α-NA, α-NB, α-NC and β-NA as substrates[a].

Substrate	Specific activity ± SE (μmol · min^{-1} · mg^{-1} protein)	
	Rp	Sa
α-NA	0.026 ± 0.009	0.084 ± 0.013*
α-NB	0.140 ± 0.004	0.236 ± 0.005*
α-NC	0.014 ± 0.000	0.039 ± 0.001*
β-NA	0.583 ± 0.008	0.783 ± 0.006*

[a] Data are the mean of three determinations, mean followed by * represent significant difference between Rp and Sa ($P < 0.05$).

https://doi.org/10.1017/S0007485309006725 Published online by Cambridge University Press

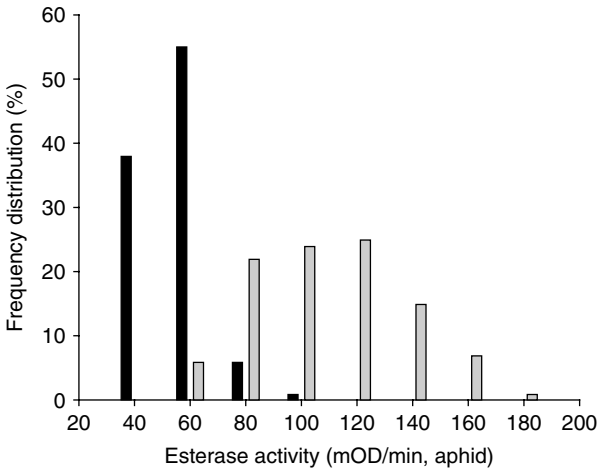

Fig.1. Frequency distributions of individual esterase activity using α-NA as substrate in *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa). The number of tested aphids was 110 aphids for each species (■, R_p; □, Sa).

Table 6. Median inhibition concentration (IC_{50}) of pirimicarb and DEF to CarE in *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa)[a].

Inhibitor	$IC_{50} \pm SE$ (M)	
	Rp	Sa
Pirimicarb	0.15 ± 0.06	$(9.18 \pm 0.49) \times 10^{-6}$*
DEF	$(3.45 \pm 1.13) \times 10^{-3}$	$(5.23 \pm 0.27) \times 10^{-4}$*

[a] Data are the mean of three determinations, mean followed by * represent significant difference between Rp and Sa ($P < 0.05$).

Table 7. Comparison of specific activity of GSTs between both *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa) using CDNB or DCNB as substrates[a].

Substrate	Specific activity ± SE (nmol · min^{-1} · mg^{-1} protein)	
	Rp	Sa
CDNB	33.71 ± 0.42	108.92 ± 1.12*
DCNB	6.20 ± 1.05	20.85 ± 2.13*

[a] Data are the mean of three determinations, mean followed by * represent significant difference between Rp and Sa ($P < 0.05$).

of two aphid species co-infesting the late stage of wheat in China.

Pirimicarb is a fast-acting, selective carbamate aphicide which is active through contact, stomach, fumigant, translaminar and root-systemic routes (Jutsum *et al*., 1988). As a neurotoxic insecticide, it could inhibit hydrolysis of neurotransmitter acetylcholine (ACh) by AChE, resulting in the disruption of normal nervous system function owing to the accumulation of acetylcholine in the synapse. The selective mechanism of pirimicarb has been reported among different aphid species (Gao & Zheng, 1989; Gao *et al*., 1990) and between *Coccinella septempunctata* and *Sitobion avenae* (Gao *et al*., 1991). Usually, the effect of the detoxification enzymes to insecticide toxicity may be revealed primarily by bioassay in the presence and absence of detoxification enzyme inhibitors. DEF and DEM are thought to be inhibitors of esterases and GSTs, respectively. PBO is accepted as an inhibitor of cytochrome P450 monooxygenases, though several reports have exhibited the inhibition of PBO on esterases activity in an opportune time after treatment with PBO (Gunning *et al*., 1999; Young *et al*., 2005, 2006; Bingham *et al*., 2008). Treatment with inhibitors of three detoxification enzymes, PBO, DEF and DEM, significantly increased toxicity of pirimicarb against these two wheat aphid species. However, the synergistic extent of three synergists on pirimicarb was approximately 2-fold higher in Sa than in Rp, indicating that esterases, cytochrome P450 monooxygenases and GSTs may contribute to the toxicity difference of pirimicarb between both aphid species. Actually, the CarE activity revealed by four substrates, α-NA, β-NA, α-NC and α-NB, and the GSTs activity conjugating with CDNB or DCNB were significantly higher in Sa than in Rp.

CarE activity and sensitivity to pirimicarb was higher in Sa than in Rp. The higher sensitivity of CarE to pirimicarb in Sa was in favor of protection of AChE from pirimicarb inhibition. This difference in CarE sensitivity between the CarE from Rp and from Sa might partly contribute to the differential susceptibilities of two species of aphids to pirimicarb. Meanwhile, the AChE was more sensitive to pirimicarb from Rp than from Sa. Therefore, the pirimicarb may be more effective for controlling Rp.

The sensitivity difference of molecular targets is one of the most important mechanisms responsible for the toxicity difference of insecticides among different animals. The sensitivity difference of AChE has been demonstrated as an important mechanism for the toxicity difference of organophosphate and carbamate insecticides among various aphids or between lady beetles and aphids (Gao & Zheng, 1989; Gao *et al*., 1990, 1991). In our studies, the IC_{50} of pirimicarb inhibiting AChE from Sa was higher than from Rp. AChE contribution to toxicity differences of pirimicarb may be based on its sensitive difference and activity difference

Table 8. Comparison of kinetic parameters of GSTs isolated from *Rhopalosiphum padi* (Rp) and *Sitobion avenae* (Sa) using CDNB and GSH as substrates[a].

Substrate	$K_m \pm SE$ (mmol L^{-1})		$V_{max} \pm SE$ (μmol · min^{-1} · mg^{-1} protein)	
	Rp	Sa	Rp	Sa
CDNB	1.30 ± 0.15	0.89 ± 0.04	0.08 ± 0.00	0.21 ± 0.02*
GSH	1.02 ± 0.12	1.05 ± 0.11	0.07 ± 0.00	0.24 ± 0.01*

[a] Data are the mean of three determinations, mean followed by * represent significant difference between Rp and Sa ($P < 0.05$).

https://doi.org/10.1017/S0007485309006725 Published online by Cambridge University Press

between both aphid species. There was no difference in AChE activity hydrolyzing ATCh, PrTCh and MeTCh in the crude extract between both aphid species. However, the significant difference of AChE activity was observed in the purified AChE. The purified AChE exhibited much lower activity to hydrolyze ButCh. This is because the enzyme in the crude extracts of AChE (table 2) is complex, including other enzymes, such as esterase, which might hydrolyze ButCh together with AChE. However, the purified AChE (table 3) can hydrolyze ATCh rapidly, and ButCh is not the optimal substrate of AChE.

Changes of AChE activity and sensitivity to inhibitor also play an important role in insect resistance to insecticide. Some researchers showed the involvement of a significant increase of AChE activity in resistance mechanisms of greenbug (*Schizaphis graminum*) (Zhu & He, 2000; Zhu *et al*., 2000; Gao & Zhu, 2002). Also, some studies on resistance mechanisms in aphids and other pests have suggested the involvement of enhanced AChE levels (Hama *et al*., 1980; Moores *et al*., 1994; Guedes *et al*., 1997; Andrews *et al*., 2004).

All the data suggested that the detoxification mechanisms (cytochrome P450 monooxygenases, esterases and glutathione S-transferases) and the difference of AChE sensitivity and activity may contribute to the toxicity difference of pirimicarb between Sa and Rp. These results should be helpful in the choice and alteration of insecticides and synergists for controlling wheat aphids.

Acknowledgements

The authors thank Dr Nannan Liu in the Department of Entomology and Plant Pathology, Auburn University for detailed discussion on the revised manuscript. This research was supported by National Basic Research Program of China (Contract No. 2006CB102003), National Key Research Program of China for the Eleventh Five-Years Plan (Contract No. 2006BAD08A03) and the National Natural Science Foundation of China (Contract No. 30530530, 30571232, 30471153, and 30170621).

References

Andrews, M.C., Callaghant, A., Field, L.M., Williamson, M.S. & Moores, G.D. (2004) Identification of mutations conferring insecticide-insensitive AChE in the cotton-melon aphid, *Aphis gossypii Glover*. *Insect Molecular Biology* **13**, 555–561.

Bingham, G., Gunning, R.V., Delogu, G., Borzatta, V., Field, L. & Moores, G.D. (2008) Temporal synergism can enhance carbamate and neonicotinoid insecticidal activity against resistant crop pests. *Pest Management Science* **64**, 81–85.

Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of proteindye binding. *Analytical Biochemistry* **72**, 248–254.

Chen, M.H., Han, Z.J., Qiao, X.F. & Qu, M.J. (2007) Resistance mechanisms and associated mutations in acetylcholinesterase genes in *Sitobion avenae* (Fabricius). *Pesticide Biochemistry and Physiology* **87**, 189–195.

Ellman, G.L., Courtey, D., Andres, V. & Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterease activity. *Biochemical Pharmacology* **7**, 88–95.

Gao, J.R. & Zhu, K.Y. (2001) An acetylcholinesterase purified from the greenbug (*Schizaphis graminum*) with some unique enzymological and pharmacological characteristics. *Insect Biochemistry and Molecular Biology* **31**, 1095–1104.

Gao, J.R. & Zhu, K.Y. (2002) Increased expression of an acetylcholinesterase gene may confer organophosphate resistance in the greenbug, *Schizaphis graminun* (Homoptera: Aphididae). *Pesticide Biochemistry and Physiology* **73**, 164–173.

Gao, X.W. (1987) Introduction of Ellman procedure for assay of cholinesterases in crude enzymatic preparations modified by Gorun. *Chinese Bulletin of Entomology* **33**, 245–246.

Gao, X.W. & Zheng, B.Z. (1989) Study on selective mechanism of pirimicarb to *Aphis gossypii* and *Myzus persicae*. *Agrochemicals* **28**, 1–2.

Gao, X.W., Wang, Z.G. & Zheng, B.Z. (1990) Selective toxicity of six common insecticides to eight species of aphids. *Acta Entomologica Sinica* **41**, 274–279.

Gao, X.W., Liu, W., Zhao, G.Y. & Zheng, B.Z. (1991) Study on selective mechanism of pirimicarb to *Coccinella septempunctata* and *Sitobion avenae*. *Agrochemicals* **30**, 40–41.

Guedes, R.N.C., Zhu, K.Y., Kambhampati, S. & Dover, B.A. (1997) An altered acetylcholinesterase conferring negative cross-insensitivity to different insecticidal inhibitors in organophosphate-resistant lesser grain borer, *Rhyzopertha dominica*. *Pesticide Biochemistry and Physiology* **58**, 55–62.

Gunning, R.V., Moores, G.D. & Devonshire, A.L. (1999) Esterase inhibitors synergise the toxicity of pyrethroids in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* **63**, 50–62.

Habig, W.H., Pabst, J. & Jackoby, W.B. (1974) Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* **249**, 7130–7139.

Hama, H., Miyata, T. & Saito, T. (1980) Some properties of acetylcholinesterase partially purified from susceptible and resistance green rice leafhopper, *Nephotettix* cincticeps (Uhler) (Hemoptera: Deltocephalidae). *Applied Entomology Zoology* **15**, 249–256.

Jutsum, A.R., Franz, J.M., Deacon, J.W., Payne, C.C., Lewis, T., Paterson, R.R.M., Waage, J.K. & van Emden, H.F. (1988) Commercial application of biological control: status and prospects. *Philosophical Transactions of the Royal Society of London, Series B* **318**, 357–373.

Liu, A.Z., Ru, T.Q., Wang, X.J. & Li, S.G. (2001) Susceptibity of both aphid species to some insecticides. *Plant Protection* **39**, 20–21.

Liu, N.N. & Yue, X. (2000) Insecticide Resistance and Cross-Resistance in the House Fly (Diptera: Muscidae). *Journal of Economic Entomology* **93**, 1269–1275.

Lu, Y.H. & Gao, X.W. (2007) A method for mass culture of wheat aphids. *Chinese Bulletin of Entomology* **44**, 289–290.

Mohammadi, S.M., Hejazi, M.J., Mohammadi, A. & Rashidi, M.R. (2007) Resistance status of the Colorado potato beetle, *Leptinotarsa decemlineata*, to endosulfan in East Azarbaijan and Ardabil provinces of Iran. *Journal of Insect Science* **7**(31) 7 pp. Available online at http://insectscience.org/7.31/.

Moores, G.D., Devine, G.J. & Devonshire, A.L. (1994) Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. *Pesticide Biochemistry and Physiology* **49**, 114–120.

Moores, G.D., Gao, X.W., Denholm, I. & Devonshire, A.L. (1996) Characterization of insensitive acetylcholinesterase in the insecticide-resistance cotton aphid, *Aphis gossypii Glover* (Homoperta : Aphidedae). *Pesticide Biochemistry and Physiology* **56**, 102–110.

Scott, J.G., Foroozesh, M., Hopkins, N.E., Alefantis, T.G. & Alworth, W.L. (2000) Inhibition of cytochrome P450 6D1 by alkynylarenes, methylenedioxyarenes, and other substituted aromatics. *Pesticide Biochemistry and Physiology* **67**, 63–71.

Shotkoski, F.A., Mayo, Z.B. & Peters, L.L. (1990) Induced disulfoton resistance in greenbugs (Homoptera: Aphididae). *Journal of Economic Entomology* **83**, 2147–2152.

Shufran, R.A., Wilde, G.E. & Sloderbeck, P.E. (1997) Response of three greenbugs (Homoptera: Aphididae) strains to five organophosphorous and two carbamate insecticides. *Journal of Economic Entomology* **90**, 283–286.

van Asperen, K. (1962) A study of housefly esterases by means of a sensitive colorimetric method. *Journal of Insect Physiology* **8**, 401–416.

Yang, X.M., Zhu, K.Y., Buschman, L.L. & Margolies, D.C. (2001) Comparative susceptibility and possible detoxification mechanisms for selected miticides in Banks grass mite and two-spotted spider mite (Acari: Tetranychidae). *Experimental and Applied Acarology* **25**, 293–299.

Young, S.J., Gunning, R.V. & Moores, G.D. (2005) The effect of piperonyl butoxide on pyrethroid-resistance-associated esterases in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pest Management Science* **61**, 397–401.

Young, S.J., Gunning, R.V. & Moores, G.D. (2006) Effect of pretreatment with piperonyl butoxide on pyrethroid efficacy against insecticide resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Bemisia tabaci* (Sternorrhyncha: Aleyrodidae). *Pest Management Science* **62**, 114–119.

Zhu, K.Y. & He, F.Q. (2000) Elevated esterase exhibiting arylesterase-like characteristics in an organophosphate-resistant clone of the greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae). *Pesticide Biochemistry and Physiology* **67**, 155–167.

Zhu, K.Y., Gao, J.R. & Starkey, S.R. (2000) Organophosphate resistance mediated by alterations of acetylcholinesterase in a resistant clone of the greenbug, *Schizaphis graminun* (Homoptera: Aphididae). *Pesticide Biochemistry and Physiology* **68**, 138–147.