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Some Biochemical Evidence on the Selective Insecticide Toxicity between the Two Aphids, *Aphis citricola* and *Myzus malisuctus* (Homoptera: Phididae), and Their Predator, *Harmonia axyridis* (Coleoptera: Coccinellidae)

Jum Rae Cho*, Young Joon Kim[1], Hong Sun Kim and Jai Ki Yoo

Department of Crop Protection, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea

[1]Division of Applied Biology and Chemistry, and Research Center for New Bio-materials in Agriculture, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Korea

Abstract This experiment was carried out to compare the differences in biochemical enzyme activity on the selective insecticide toxicity between the two species of aphid, *Aphis citricola* van der Goot and *Myzus malisuctus* Matsumura, and their predator, *Harmonia axyridis* Pallas. Esterase activities between the two species of aphids and between the two stages of *H. axyridis* were significant different. Glutathione *S*-transferase (GST) activity toward 1-chloro-2, 4-dinitrobenzene (CDNB) was much higher than 1, 2-dichloro-4-nirobenzene (DCNB) in all species tested. No DCNB conjugation was detected in *A. citricola* and *M. malisuctus*. The predator, *H. axyridis*, had much higher GST activity than the preys, *A. citricola* and *M. malisuctus*. GST activity toward CDNB in *H. axyridis* adult was highest, even 6.2-fold higher activity than *H. axyridis* larva. *M. malisuctus* had much higher GST activity than *A. citricola*. The degree of acetylcholinesterase (AChE) inhibition by phosphamidon among all three species tested was significantly varied. The concentration of phosphamidon required for 50% AChE inhibition was lowest in *H. axyridis* larva, while highest in *M. malisuctus*. Therefore, elevated GST activity and target-site insensitivity may be largely associated with the differential susceptibility between larva and adult of *H. axyridis*. However, differential susceptibility between *A. citricola* and *M. malisuctus* may be due to other various biochemical mechanisms responsible for the multiple selective toxicity, including elevated GST activity and target-site insensitivity.

Key words *Aphis citricola*, *Myzus malisuctus*, *Harmonia axyridis*, selective toxicity, detoxification enzyme

*Corresponding author.
E-mail: jrchol@rda.go.kr
Tel: 031-290-0464; Fax: 031-290-0479

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Introduction

Among 312 species of apple insect pests in Korea, the most important species are two aphids, spiraea aphid, *Aphis citricola* van der Goot, and apple leaf-curling aphid, *Myzus malisuctus* Matsumura (Anonymous, 1986; Lee, 1990). The aphidophagous coccinellid, *Harmonia axyridis* Pallas, is an important predator of several crop pests, particularly in apple orchards. Conservation and use of this predator are important components in integrated pest management programs. To preserve this natural enemy population, it will be essential to use the selective insecticides that are toxic against insect pests but relatively safe for the predators (Yu, 1988; Cho *et al.*, 1997).

Several researchers have reported the differential insecticide susceptibility between the prey and parasite or predator (Bartlett, 1966; Lindgren *et al.*, 1972; Koehler *et al.*, 1993). This differential susceptibility may result from the biochemical differences in the ability to detoxify insecticides between the prey and its parasite or predator. Comparative studies of detoxification enzyme systems between the prey and its parasite or predator are greatly lacking (Mullin *et al.*, 1982; Mullin and Croft, 1984; Yu, 1987, 1988; van de Baan, 1988).

Cho *et al.* (1997) reported that almost all insecticide tested were more toxic to the pest than to the predator and alphamethrin showed the lowest selectivity ratio for larvae and adults of *H. axyridis* compared with *A. citricola* and *M. malisuctus*. They suggested that lower susceptibility of *H. axyridis* to the synthetic pyrethroid might be due to the high levels of detoxifying enzyme activities. Yu (1987, 1988) examined selectivity relationships between predator and its prey comparing detoxification enzyme systems. Yu (1987) reported that the predator showed more enzyme activity than its prey. Bioassay results showed that

the predator was generally susceptible to organophosphate and carbamate insecticides than the prey. Yu (1988) concluded that these differences probably were due to the lesser detoxification capabilities of the beneficial species. Pyrethroid and carbamate insecticides can be metabolized by microsomal oxidases and esterases in insects (Shono *et al.*, 1979; Kuhr and Hessney, 1977), whereas the organophosphate can be metabolized by microsomal oxidases as well as glutathione s-transferase (Yang *et al.*, 1971). However, the exact mechanisms on differential insecticide toxicity between insect pests and their natural enemies are as yet unknown. Therefore, this present study was carried out to analyze the biochemical enzyme activity on the selective insecticide toxicity between the two species of aphids, *A. citricola* and *M. malisuctus*, and the predator, *H. axyridis*.

Materials and Methods

Insects and chemicals

The spiraea aphid, *Aphis citricola*, and apple leaf-curling aphid, *Myzus malisuctus*, were collected from an apple orchard of Suwon in 1998. The aphidophagous coccinellid, *Harmonia axyridis*, was collected from the rose of Sharon, *Hibiscus syriacus* L., in Suwon in 1998. Phosphamidon (2-chloro-2-diethyl carbmoyl-1-methyl vinyl dimethylphosphate, 90%) used for inhibition of acetylcholinesterase was purchased from a local commercial supplier. All chemicals used for enzyme assay were of reagent grade or better.

Enzyme assay

Test insects of each species were homogenized in 0.2 M phosphate buffer (pH 8.0) with 0.2 % Triton X-100. After centrifugation (12,000*g* x 10 min, 4℃), the supernatant was used as an enzyme source. Protein concentration was determined by the method of Bradford (1976).

The rate of degradation of α- and β-naphthyl acetate (NA) (Sigma Chemical Co., Mo, USA) was measured by the method of van Asperen (1962) with slight modifications. Each of twenty-five aphids and one predator was homogenized in 0.5 ml of 4 mM ice-cold potassium phosphate buffer (pH 6.8) with 0.05% Triton X-100. The homogenate was centrifuged at 1,000 g for 20 min. The supernatant fraction (0.05 ml) and 0.004 M potassium phosphate buffer (pH 6.8, 0.45 ml) were mixed, and incubated in an effendorf tube for 15 min at 37℃. After incubation, 0.5 ml of 0.5 x 10^{-3} M α- and β-NA in ethanol (final concentration, 2.5 x 10^{-4} M) were added to the mixture. The mixture was re-incubated for 15 min in α- NA and 5 min in β-NA at 37℃. The reaction was stopped and color developed by adding 0.5 mℓ dye solution (1 % diazoblue B salt: 5 % sodium lauryl sulfate = 2: 5, v/v). After incubation for 20 min, the absorbance was determined at 600 nm for α-naphthol and at 550nm for β-naphthol using a spectrophotometer (Ultrospec2000, Pharmacia Biotech Co.).

Glutathione *S*-transferase (GST) activities were spectrophotometrically assayed with the soluble fraction (105,000g supernatant) as the enzyme source. GST activity toward 1-chloro-2,4-dinitrobenzene (CDNB) was determined using a 1.5 ml of reaction mixture containing 0.01-0.02 μg protein, 0.1 M potassium phosphate buffer (pH 6.5), 1 mM reduced glutathione (GSH), and 1 mM CDNB in ethanol. Toward 1, 2-dichloro-4-nirobenzene (DCNB), reaction mixture consisted of 0.4-1.0 μg protein, 0.1 M Tris-HCl buffer, pH 9.0, 5 mM reduced GSH, and 1 mM DCNB. Absorbance was recorded at 340 nm for CDNB and 344 nm for DCNB. GST activity was expressed as nM hydrolyzed substrate/min/mg protein by using the extinction coefficient of 8.5 $mM^{-1}cm^{-1}$ for the DCNB conjugate and 9.6 $mM^{-1}cm^{-1}$ for the CDNB conjugate (Habig *et al.*, 1974).

Acetylcholinesterase (AChE) activity was measured by the method of Ellman *et al.* (1961). For inhibition experiments, phosphamidon solutions were prepared by dissolving the insecticide in 10 ml of acetone for appropriate dilution. Homogenate was pre-incubated with phosphamidon for 10 min before the substrate was added. Readings were taken every 2 min for 30 min after addition of substrate. The absorbance at 10 min was used for calculations. The inhibition of AChE activity was determined by comparing the activity in treated homogenates with that in uninhibited controls. Concentration of phosphamidon for 50 % inhibition of AChE activity was estimated by probit analysis (Raymond, 1985).

Results and Discussion

Detoxifying enzyme activities in *A. citricola*, *M. malisuctus*, and *H. axyridis*, are shown in Table 1. Esterase activities toward α-naphthyl acetate (NA) were a slightly higher in *A. citricola* than in other species, whereas the activities toward β-naphthyl acetate (NA) were a slightly higher in *M. malisuctus* than in the other species. Esterase activities between the two species of aphid and between the two stages of *H. axyridis* were significant different.

Differential toxicity of insecticides has been related

Table 1. The activity of detoxification enzymes in *Aphis citricola, Myzus malisuctus,* and the predator *Harmonia axyridis*
Insect species esterase activity

Species	Esterase activity (μmol/min/mg protein)		Glutathione S-transferase activity (nmol/min/mg protein)	
	α-NA	β-NA	CDNB	DCNB
A. citricola	0.30±0.02a	0.27±0.02b	4.1±0.84d	ND[a]
M. malisuctus	0.10±0.01d	0.50±0.02a	9.5±0.97c	ND[a]
H. axyridis larva	0.22±0.03b	0.22±0.04c	82.6±11.33b	1.1±0.33a
H. axyridis adult	0.19±0.02c	0.20±0.02d	511.6±17.82a	0.7±0.01a

Means followed by the same letter within a column are not significantly different at P = 0.05 (Scheffe's test).
[a] Not detectable.

Table 2. The inhibition of acetylcholinesterase by phophamidon in *Aphis citricola, Myzus malisuctus,* and the predator *Harmonia axyridis*

Insect species	I_{50}[a] (95% CL)	Slope (±SE)	χ^2	df
A. citricola	0.22 (0.15-0.29)	1.43 (0.16)	17.34	1
M. malisuctus	452.64 (193.67-2051.77)	0.41 (0.09)	4.7	1
H. axyridis larva	0.02 (0.01-0.05)	0.37 (0.05)	0.69	1
H. axyridis adult	3.67 (2.36-5.37)	0.98 (0.11)	8.42	1

[a] Inhibition concentration expressed in μM. I_{50} for each insect is considered significant when 95% CL levels failed to overlap.

to various factors such as reduced penetration, enhanced metabolic degradation, and altered target site (Oppenoorth and Welling, 1976). Stage-dependent insecticide tolerance that is a common phenomenon in insects is often correlated with enhanced detoxification enzyme activity (Yu, 1983; Christie and Wright, 1990; Koehler *et al.*, 1993). Christie and Wright (1990) reported that the enhanced oxidative detoxification and reduced penetration might be responsible for the differential susceptibility in *Spodoptera littoralis* (Boisd.). Valles *et al.* (1994) reported no significant differences in general esterase, carboxylesterase, and GST among different stages of the German cockroach (*Blatella germanica* L.). Valles *et al.* (1996) concluded that the stage-dependent propoxur tolerance in the German cockroach was largely due to enhanced microsomal oxidation, with reporting no marked difference in the rate of penetration of propoxur in nymphs and adults. Plapp and Bull (1978) found that pyrethroid insecticides were highly toxic to the tobacco budworm but very low in toxicity to the predator, common green lacewing. Zhuravskaya *et al.* (1976) found that penetration of the organophosphate phosmet was faster in the cotton aphid *Aphis gossypii* than in either the adult or the third larval instar of *Chrysopelrla carnea*. These stages of the predator were 49 and 190 times more tolerant to the pesticide than the aphid. They concluded that the observed difference in rates of penetration was a significant factor for the selective action of the insecticide. Mullin *et al.* (1982) compared detoxification enzymes in the polyphagous spider mite *Tetranychus urticae* and its major acarine predator *Amblyseius fallacis*. They found that major enzyme activity differences were closely associated with patterns of susceptibility observed among strains. Comparing pesticide susceptible strains, the herbivore had 5-fold higher mixed function oxidase (MFO) and 6-fold higher transepoxide hydrolase levels than the carnivore. Also, the herbivore and carnivore had similar esterase activities for α-naphthyl acetate. In the present study, significant difference in esterase activity between the two species of aphid and between the two stages of *H. axyridis* may be associated with the selective insecticide toxicity obtained by Cho *et al.* (1997) among three species.

GST activity toward CDNB was much higher than toward DCNB in all species tested. Of all species tested, GST activity toward CDNB in *H. axyridis* adult was highest, even 6.2-fold higher activity than *H. axyridis* larva. No DCNB conjugation was detected in *M. malisuctus* and *A. citricola*. The reason why DCNB conjugation was not detected in *M. malisuctus* and *A. citricola* is unclear. *M. malisuctus* had much higher GST activity than *A. citricola*. The predator, *H. axyridis*, has much higher GST activity than the prey, *A. citricola* and *M. malisuctus*. This result is similar pattern to the finding of Mullin *et al.* (1982) reported that the predator, *Amblyseius fallacis* had 11-fold higher GST activities than the prey, *Tetranychus urticae* (Koch). Hung *et al.* (1990) reported that GST activity toward CDNB in all the tested insects was much higher than DCNB; no DCNB conjugation was detected in *Nilaparvata lugens* (Stål) and *Laodelphax striatellus* (Fallén). Yu (1987) reported that the predator was generally more susceptible to organophosphate

and carbamate insecticides than the prey. Yu (1988) concluded that differential toxicity between the prey and predator probably was due to the lesser detoxification capabilities of the beneficial species. In this study, elevated GST activity in the predator may be associated with the result of Cho *et al.* (1997) that the predator *H. axyridis* toward pyrethroid and organophosphate insecticides was generally higher tolerant than the prey. Therefore, comparative studies on toxicity and detoxification between arthropod natural enemies and their phytophagous preys suggest numerous possibilities for exploiting physiological, biochemical, or toxicological differences between the two groups to obtain selectivity in future pesticides.

The inhibition of AChE by phosphamidon in *A. citricola, M. malisuctus*, and *H. axyridis*, is shown in Table 2. The inhibition rate of AChE by phosphamidon among all species tested was significantly different. Phosphamidon, which showed the highest selectivity ratio, was less safe to the predator than to the pest (Cho *et al.*, 1997). The concentration of phosphamidon required for AChE inhibition in *H. axyridis* adult was 183.5-fold higher than that in *H. axyridis* larva. Higher I_{50} values in *H. axyridis* adult demonstrate that AchE insensitivity may be associated with the enhanced tolerance of *H. axyridis* adult to organophosphate insecticides. However, higher levels of GST activity and I_{50} values in *M. malisuctus* than in *A. citricola* could not confirm that result of Cho *et al.* (1997) that *M. malisuctus* was much more susceptible than *A. citricola.*

In conclusion, elevated GST activity and target-site insensitivity may be associated with the differential susceptibility between larva and adult of *H. axyridis*. However, differential susceptibility between *A. citricola* and *M. malisuctus* may be due to other various biochemical mechanisms responsible for the multiple selective toxicity, including elevated GST activity and target-site insensitivity. More exact mechanism remains to be clarified.

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