SHORT COMMUNICATIONS

A Fenitrothion-Insensitive Mechanism of the Caddisfly, *Cheumatopsyche brevilineata* (Trichoptera: Hydropsychidae), a Dominant Species in Pesticide-Polluted Rivers¹

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From the faunal investigations of macrobenthos in several rivers, it has become apparent that one species of Trichoptera (caddisfly), *Cheumatopsyche brevilineata*, and several kinds of Chironomids (midges), such as *Chironomus yoshimatsui*, *Glyptotendipes tokunagai* and *Dicortendipes nerbosus*, are representative dominant species in aquatic environments heavily polluted with insecticides (HATAKEYAMA, 1991; SUGAYA, unpublished data). To clarify the mechanisms of this adaptation, toxicity of several insecticides which have been detected in rivers was examined. As might be expected, the aquatic species were remarkably insensitive to organophosphorus (OP) insecticides and carbamates such as fenitrothion and BPMC (HATAKEYAMA, unpublished data). However, details of the mechanisms of this insensitivity to insecticides are not clear, except in mosquitoes (GEORGHIOU and MELLON, 1983). We report here the mechanisms of the insensitivity to fenitrothion in *C. brevilineata*.

MATERIALS AND METHODS

The following three fenitrothion-insensitive strains (Suna, Mitsu and Tama) of *C. brevilineata*

were used in this study. Fourth instar larvae of the Suna strain (body weight: 9 to 11 mg) were collected from a sampling station of the River Suna in Takahata, Yamagata Prefecture, in September 1991. Fourth instar larvae of the Mitsu strain (body weight: 9 to 11 mg) were collected from a paddy field irrigation ditch in Mitsukaido, Ibaraki Prefecture, in August 1992. Fourth instar larvae of the Tama strain (body weight: 9 to 11 mg) were collected from the Tama Channel, Higashi-Yamato, Tokyo in September 1992.

Because we could not find an insecticide-susceptible strain of *C. brevilineata*, we used *Cheumatopsyche* sp. as reference standard organisms for the evaluation of the biochemical data obtained from *C. brevilineata*. *Cheumatopsyche* sp. is recently separated from *C. brevilineata* as a new species on the basis of a difference in head capsule shape (i.e. the former's is elongated in contrast to the round shape of the latter) (TANIDA, pers. comm.). Fourth instar larvae of *Cheumatopsyche* sp. (body weight: 9 to 11 mg) were collected from a small mountain stream (Segami Creek, where insecticides had not yet been applied) in Yokohama, Kanagawa Prefecture, in October 1991. These materials were transferred to the laboratory by keeping them in river water enriched with oxygen. The following acute toxicity test of the insecticides and the biochemical experiments were conducted on the day after collection.

Fenitrothion (*O,O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothioate, 99.4% purity), POPOP (1,4-bis[2-(5-phenyloxazolyl)]benzene) and PPO (2,5-diphenyloxazole) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fenitroxon (*O,O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphate, 99.2% purity) and [Methoxy-¹⁴C]fenitroxon (5.92 MBq/mmol, 99.1% purity) were provided by Sumitomo Chemical Co., Ltd. (Osaka, Japan). NCS-II, a tissue solubilizer, was obtained from Amersham Co. (Ill, USA). All other chemicals were purchased from commercial sources.

Seven fourth instar larvae individuals were introduced to a 100 ml glass beaker filled with ca. 70 ml of artificially prepared soft water (ASW) (TABATA, 1972). On the next day, abnormal individuals (judged by the nest spun with silk thread) were removed from the beaker. ASW

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(100 ml) was renewed, and the insecticides dissolved in ethanol were added to each beaker to make concentration series of the insecticides for the acute toxicity test. The test was conducted with three replicates per concentration, and ethanol concentration was adjusted to 0.1% at every concentration including control. LC_{50} values were calculated by probit method based on mortality 24 h after the treatments.

Enzymes were prepared as follows. The whole bodies of larvae were homogenized (20% w/v) with 1 mM EDTA-5 mM 2-mercaptoethanol-0.1 M phosphate buffer (EMP buffer), pH 7.4, in an ice bath. The homogenate was centrifuged at 600 *g* for 10 min to remove nuclei and debris. The particulate fraction (105,000 *g* precipitate) and soluble fraction (105,000 *g* supernatant) were separated from the 600 *g* supernatant by centrifugation. The particulate fractions (mitochondria + microsomes) were suspended in EMP buffer.

The acetylcholinesterase (AChE) activity and its sensitivity to fenitroson were determined by the method of ELLMAN et al. (1961) with acetylthiocholine iodide as substrate. Because more than 80% of AChE activity was recovered in the particulate fraction both from *C. brevilineata* and *Cheumatopsyche* sp., the particulate fraction was used as the enzyme source. The reaction mixture consisting of 0.1 ml of the enzyme source, 2.8 ml of 0.05 M phosphate buffer, pH 8.0, 0.2 ml of 3 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and 0.01 ml of fenitroson (ethanol solution) was preincubated at 25°C for 5 min, then mixed with 0.02 ml of 0.1 M acetylthiocholine iodide. AChE inhibition

by fenitroson was calculated from the changes of the absorbance of the reaction mixture at 412 nm.

Fenitroson detoxication by binding protein was measured according to the method of KONNO and SHISHIDO (1989). A 1-ml quantity of the enzyme source (1 mg protein/ml) was incubated at 27°C for up to 1 h with 1 μ g of [Methoxy- 14 C]fenitroson dissolved in 10 μ l of ethanol in the presence (non-specific binding) or absence (total binding) of 100 μ g unlabeled fenitroson. After an appropriate time, 1 ml of 14% trichloroacetic acid (TCA) was added to the mixture to precipitate protein, and then 3 ml of $CHCl_3$ was added to remove unchanged fenitroson. The protein precipitate was filtered off, washed with TCA and $CHCl_3$ and solubilized with 1 ml of NCS-II, and radiocarbon bound to protein was measured by liquid scintillation counting (LSC) with a toluene scintillator (toluene:POPOP=997:3). The specific binding was calculated by subtracting nonspecific binding from total binding. Data are the mean of three experiments.

Fenitroson detoxication by hydrolysis was measured according to the method of KONNO and SHISHIDO (1989). A 1 ml quantity of the enzyme source (1 mg protein/ml) was incubated at 27°C for up to 1 h with 1 μ g of [Methoxy- 14 C]fenitroson dissolved in 10 μ l of ethanol. After an appropriate time, 1 ml of 14% trichloroacetic acid (TCA) was added to the mixture to precipitate protein, and then 3 ml of $CHCl_3$ was added to remove unchanged fenitroson. Radiocarbon of the water-soluble fraction possibly resulting from enzymatic hydrolysis was measured by LSC with a dioxane

Table 1. Toxicity of fenitrothion to the three strains of *Cheumatopsyche brevilineata* and *Cheumatopsyche* sp.

Species	LC_{50} (ppm) ^a	Insensitivity ratio ^b
<i>C. brevilineata</i>		
Suna strain	4.35 (2.67–7.10)	218
Mitsu strain	5.07 (3.33–11.98)	254
Tama strain	1.30 (1.10–1.83)	65
<i>Cheumatopsyche</i> sp.	0.02 (0.014–0.024)	1

^a Toxicity was evaluated at 24 h. Figures in parentheses indicate 95% confidence limits.

^b *Cheumatopsyche* sp.=1

scintillator (dioxane : naphthalene : PPO=950:45:5). For the control of nonenzymatic hydrolysis, reaction mixtures without enzyme sources were used. Data are the mean of three experiments. Protein concentration was determined according to the method of BRADFORD (1976).

RESULTS AND DISCUSSION

The susceptibility of the three strains of *C. brevilineata* and *Cheumatopsyche* sp. to fenitrothion is shown in Table 1. The three strains of *C. brevilineata* were from 65 to 254 times less sensitive to fenitrothion than *Cheumatopsyche* sp.

The median inhibition concentrations (I_{50}) of fenitrothion against AChE are shown in Table 2. The AChEs of *C. brevilineata* were rather more sensitive to fenitrothion than that of *Cheumatopsyche* sp. This indicates that the insensitivity of *C. brevilineata* to fenitrothion is not attributed to the

insensitivity of AChE to fenitrothion.

As in the rice stem borer, *Chilo suppressalis* (KONNO and SHISHIDO, 1989) and the cotton aphid, *Aphis gossypii* (SUZUKI et al., 1993), fenitrothion detoxication activity in *C. brevilineata* and *Cheumatopsyche* sp. existed only in the soluble fraction. Time course of protein binding and hydrolysis of fenitrothion in the soluble fraction of the three strains of *C. brevilineata* and *Cheumatopsyche* sp. are shown in Fig. 1. In the three strains of *C. brevilineata*, a considerable amount of radiocarbon was detected in the protein precipitate. The radiocarbon recovered from the protein precipitate almost reached an equilibrium state at zero-time incubation, and the equilibrium state continued up to 60 min. In *Cheumatopsyche* sp., however, the fenitrothion detoxication activity by binding protein was markedly low (Fig. 1). The binding activity of *Cheumatopsyche* sp. (24.5 pmol/mg protein/60 min)

Table 2. AChE sensitivity to the inhibition by fenitrothion in the three strains of *Cheumatopsyche brevilineata* and *Cheumatopsyche* sp.

Species	I_{50} (M)	Insensitivity ratio ^a
<i>C. brevilineata</i>		
Suna strain	7.4×10^{-6}	0.82
Mitsu strain	1.2×10^{-6}	0.13
Tama strain	2.1×10^{-6}	0.23
<i>Cheumatopsyche</i> sp.	9.0×10^{-6}	1.00

^a *Cheumatopsyche* sp. = 1.00

Fig. 1. Time course of hydrolysis and protein binding of fenitrothion incubated with each soluble fraction from the Suna (●), Mitsu (△) and Tama (×) strains of *Cheumatopsyche brevilineata* and *Cheumatopsyche* sp. (○).

was 10 to 14 times lower than that of the three strains of *C. brevilineata* (248–337 pmol/mg protein/60 min).

On the other hand, hydrolysis of fenitroxon was very weak, and similar, in the two species. This experimental evidence leads us to conclude that the high activity of the fenitroxon detoxication by binding protein in the soluble fraction should be one of the mechanisms of the insensitivity to fenitrothion in *C. brevilineata*.

The role of esterases as binding protein or "sequestration" to protect AChE from inhibition by oxygen analogs of OP insecticides have been reported with the green rice leafhopper, *Nephotettix cinctipes* (HOSOKAWA and MOTOYAMA, 1981; MOTOYAMA et al., 1984) and the peach-potato aphids, *Myzus persicae* (DEVONSHIRE and MOORES, 1982). KONNO and SHISHIDO (1989) and SUZUKI et al. (1993) also reported that the fenitroxon binding by some protein in the soluble fraction was the principal mechanism of fenitrothion resistance in the rice stem borer and the cotton aphid, respectively. However, the present study is the first evidence that an aquatic insect also can protect AChE from inhibition by oxygen analogs of OP insecticides by binding protein in the soluble fraction.

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REFERENCES

- BRADFORD, M. M. (1976) *Analyt. Biochem.* **72**: 248–254.
- DEVONSHIRE, A. L. and G. D. MOORES (1982) *Pestic. Biochem. Physiol.* **18**: 235–246.
- ELLMAN, G. L., K. D. COURTNEY, V. ANDRES and R. M. FEATHERSTONE (1961) *Biochem. Pharmacol.* **7**: 88–95.
- GEORGHIOU, G. P. and R. B. MELLON (1983) *Pest Resistance to Pesticides* (G. P. GEORGHIOU and T. SAITO, eds.). Plenum Press, New York, pp. 1–46.
- HATAKEYAMA, S. (1991) *Annual Report of NIES Research Projects*, AR-4-'91, p. 62.
- HOSOKAWA, M. and N. MOTOYAMA (1981) *Annual Meeting of Pestic. Sci. Soc. Japan*, Nagoya.
- KONNO, Y. and T. SHISHIDO (1989) *J. Pesticide Sci.* **14**: 359–362.
- MOTOYAMA, N., L. R. KAO, P. T. LIN and W. C. DAUTERMAN (1984) *Pestic. Biochem. Physiol.* **21**: 139–147.
- SUZUKI, K., H. HAMA and Y. KONNO (1993) *Appl. Entomol. Zool.* **28**: 439–450.
- TABATA, K. (1972) *J. Water Waste.* **14**: 1297–1303.

Partial Rearing of a Phytophagous Lady Beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae)¹

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The phytophagous lady beetle, *Epilachna vigintioctopunctata*,

is an oligophagous insect which feeds only on solanaceous plant leaves. Only a few reports have been published on the rearing of phytophagous beetles, including this species, on an artificial diet. WARDOJO (1969) and HSIAO and FRAENKEL (1968) reared the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, on artificial diets. KOGAN (1971) reared the larvae of the Mexican bean beetle, *Epilachna varivestis*, on artificial semiliquid diets. The diets used for the rearing of these species, however, could not support the entire larval growth.

KONO (1980) showed that *E. vigintioctopunctata* could be reared on sliced potato tubers as an alternative diet, but that the development of the fat body would be delayed.

The development of a suitable artificial diet for

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