Ghana J. Sci. **39**, 97 - 101

STUDIES ON SOME ESTERASES OF *COCCINELLA SEPTEMPUNCTATA* BRUKII (COLEOPTERA: COCCINELLIDAE) WITH VARYING DEGREES OF INSECTICIDE TOLERANCE, AND IMPLICATIONS FOR INTEGRATED MANAGEMENT OF APHIDS

E. O. Owusu

Department of Zoology, Faculty of Science, University of Ghana, P.O. Box 67, Legon, Ghana

Abstract

The use of insecticide resistant strains of the predator beetle, *Coccinella septempunctata* Brukii (Coleoptera: Coccinellidae) in integrated control of aphids looks promising. However, before a true understanding of the extent of resistance can be obtained, it is important to know the mechanisms involved. Consequently, studies on some esterases of *C. septempunctata* were conducted. The aim was to find out if any relationship exists between esterase activities and insecticide tolerance in this insect. Results revealed that acetylcholinesterase and carboxylesterase activities are positively linked to insecticide (both dichlorvos and permethrin) tolerance. Again, studies on some basic properties of 2-naphthyl acetate linked carboxylesterase showed that enzymes from the most tolerant (LBB-R) and most susceptible (LBB-S) strains were mostly located in the soluble fraction and had equal activity peaks at 50 °C, and 2.5×10^{-3} *M* of substrate concentration, respectively. Peak *p*H activities, however, differed and were 6.8 for LBB-S and 7.5 for LBB-R.

Introduction

The predator lady bird beetle, *Coccinella septempunctata* Brukii (Coleoptera: Coccinellidae), is an important insect used in integrated management of aphids. However, continued use of insecticides to control aphids and other pests has resulted in high mortality of this beneficial insect. This is because most of the target organisms (aphids) have developed resistance and as a result the frequency and dosage of insecticide application have increased considerably. To help circumvent the problem of indiscriminate killing of such beneficial insects, the possibility of using insecticide resistant strains of the beetle in cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), resistant management programme was explored.

Before a thorough understanding of the extent of resistance could be obtained, however, it is pertinent to study the underlying mechanisms responsible. For this reason, the relationship between insecticide tolerance and esterase activity in species of *C. septempunctata* was investigated. Esterase was chosen as the first enzyme system to be investigated because it is the main mechanism responsible for resistance in *Myzus persicae* Sulz. (Homoptera: Aphididae) (Devonshire, 1973; Devonshire & Sawicki, 1979; Devonshire & Moore, 1982) and *A. gossypii* (Hama & Hosoda, 1988; O'Brien *et al.*, 1992; Owusu, Horiike & *Hinano* 1996a,b), two major aphid pests that are predated upon by *C. septempunctata.*

Experimental

Insects

An insecticide-resistant strain of *C. septempunctata* was collected from the field and repeatedly selected for increasing resistance with dichlorvos. The resulting resistant strain was named LBB-R. Strain LBB-S (the most susceptible

strain) was collected from an insecticide-free location and kept in the laboratory for nearly 4 years without exposure to insecticides. At the time of experiment, the LBB-R strain had 17- and 14- fold resistance to dichlorvos and permethrin, respectively.

Enzyme preparations

Ten adult females of *C. septempunctata* strains were homogenized in 2 ml of potassium phosphate buffer containing 0.2 per cent of Triton × -100. The crude homogenate was filtered through nylon cloth to exclude the debris, and topped up with buffer to 3 ml before centrifuging for 20 min at 3000 g. The resultant supernatant fraction was used as enzyme source for assay.

Enzyme assays

Assay procedures for carboxyleseterase, cholinesterase and *p*-nitrophenyl acetate hydrolyzing esterase have been described in previous papers (Owusu *et al.,* 1994; Owusu, 1995; Owusu & Adjei-Afriyie, 1998.)

Protein determination

Protein contents of all enzyme preparations used were determined by the original method of Lowry *et al.* (1951), as modified and adopted by Owusu *et al.* (1994).

Temperature, pH, substrate concentration and subcellular activity distribution

These studies were carried out with both LBB-S and LBB-R strains. Seven temperature, eight *p*H and nine substrate (2-naphthyl acetate) levels were used. The method used was exactly as described for carboxylesterase (Owusu *et al.,* 1994).

For subcellular distribution of enzyme activity, approximately 200 mg of insects was homogenized in 0.01 *M* phosphate buffer (*p*H 7.0) containing 0.25 *M* sucrose and filtered to remove the debris. One-third of the filtrate was designated as crude homogenate. The other two-thirds was divided into four subcellular fractions (Owusu *et al.*, 1994; Owusu & Horrike, 1997). A Himac SCR-20B refrigerating centrifuge was used for centrifugation up to 10,000 g while Beckman L6-70M centrifuge was used for that at 100,000 g. Enzyme activities of all subcellular fractions, as well as crude homogenate at the same dilution rate, were determined as described by Owusu *et al.* (1994).

Results and discussion

Tables 1 and 2 relate the level of insecticide tolerance to esterase activities in strains of *C. septempunctata.* High correlation was observed between dichlorvos/permethrin tolerance and acetylcholinesterase/carboxylesterase levels. This suggests that these two enzyme systems may be involved in insecticide resistance of this insect. The role of esterases in insecticide toxicology in arthropods has been extensively reviewed (Devonshire, 1973; Dauterman, 1983; Oppenoorth, 1984). In *A. gossypii* and *M. persicae* (two aphid hosts of *C. septempunctata*), carboxylesterase has been documented as the main resistance mechanism (Devonshire, 1973; Devonshire & Sawicki, 1979; Hama & Hosoda, 1988; O'Brien *et al.,* 1992; Owusu *et al.*, 1996a,b). However, the role of acetylcholinesterase is yet to be clearly defined in any of these two aphid species. With predator and its host aphids possibly possessing different mechnisms of insecticide tolerance, continued selection by insecticides may result in truly-resistant strains of *C. septempunctata* that could be used for on-farm integrated aphid control.

Evolution of pesticide resistance in arthropod natural enemies has so far provided mixed results. Out of the few arthropod predators or parasites that have been tested to determine whether they have acquired pesticide resistance under field conditions, only a few species have been shown to possess acquired resistance to pesticides (Croft, 1975). Generally, arthropod natural enemies are perceived to be slow or unlikely to develop pesticide resistance in either the laboratory or the field due to a combination of biological, ecological and/or biochemical reasons. This, however, is still not confirmed and remains open to debate (Hoy, 1990).

TABLE 1
Level of insecticide tolerance and measured esterase activities in strains of C. septempunctata

Clone	*LC_{50} (ppm)*		*CarE activity*		*ChE activity*		*p-NpE activity*
	Dichlorvos	*Permethrin*	*1-NaA*	*2-NaA*	*ASCh*	*BSCh*	p-*NaA*
LBB-1	89.2	321.3	26.5	37.2	0.59	0.65	0.48
LBB-2	153.8	396.3	72.7	69.3	0.62	0.55	0.64
LBB-3	92.3	405.3	59.2	75.2	0.55	0.62	0.72
LBB-4	99.9	562.7	68.9	72.7	0.52	0.71	0.32
LBB-5	102.2	352.7	70.2	71.1	0.57	0.82	0.47
LBB-6	169.4	459.8	75.8	79.2	0.73	0.77	0.65
LBB-7	78.8	286.7	67.3	70.2	0.44	0.62	0.59
LBB-8	108.7	277.8	72.5	74.3	0.61	0.71	0.48
LBB-9	98.2	407.9	65.3	66.2	0.52	0.54	0.66
LBB-10	84.8	463.8	65.3	64.7	0.62	0.42	0.72
LBB-S	22.5	42.9	22.5	26.8	0.52	0.59	0.51
LBB-R	378.8	598.9	88.5	86.6	0.92	0.81	0.67

Carboxylesterase (CarE) activity = nmol/10min/μg protein

Cholinesterase (ChE) & *p*-nitrophenyl acetate hydrolyzing esterase (*p*-NpE) activities=mmol/min/mg protein

TABLE 2
Two-way table showing regression coefficients (r; P= *0.001*) *of insecticide-esterase relationship in strains of* C. septempunctata.

	1-NaA	*2-NaA*	*ASCh*	*BSCh*	p-*NpE*
Dichlorvos	0.644	0.606	0.906	0.495	0.302
Permethrin	0.719	0.748	0.538	0.225	0.193

The lack of ability to detoxify secondary plant compounds as efficiently as phytophagous insects is seen as a major reason. However, the type of pesticide used, its persistence period, the type of secondary componds (formed either in storage or after exposure to external conditions), as well as the inherent potential of the particular predator/parasite to possess resistance gene, in its gene pool may be factors that could influence arthropod predators/parasites to develop some defence mechanisms. The present results, nonetheless, support the view that some arthropod predators may be capable of developing, to an extent, some forms of defence mechanisms that could be of interest in integrated pest management.

Subcellular distribution study on esterase hydrolyzing 2-naphthyl acetate indicated that in both the highly-tolerant and most-susceptible strains, bulk of enzyme activity occurred in the soluble fraction (Table 3). The amounts located in the soluble fractions of both strains were, however, less than the 82 per cent of specific activity (1-naphthyl acetate) for *A. gossypii* (Owusu *et al.*, 1994). Also, working with organophosphate-resistant *Nephotetiix cincticeps* Uhler (Hemiptera: Cicadellidae), Motoyama *et al.* (1984) found that 74 per cent of *p*-nitrophenyl acetate hydrolyzing

TABLE 3
Subcellular distribution of 2-naphthyl acetate hydrolyzing carboxylesterase in homogenates of C. septempunctata *strains LLB-S and LBB-R*

Fraction	*Percent of total activity*		*Percent of specific activity*	
	LBB-S	*LLB-R*	*LBB-S*	*LLB-R*
Crude	45.45	47.30	28.25	25.35
470 *g* (pellet)	6.55	7.83	8.42	7.88
10000 *g* (pellet)	2.33	2.14	3.80	3.84
100000 *g* (pellet)	2.11	2.14	3.80	3.84
100000 *g* (supernatant)	43.55	40.59	55.32	58.39

esterase activity was located in the soluble fraction. Even though the values obtained in the present study were lower than other reported cases, they, nonetheless, show that the majority of aromatic substituted acetate hydrolyzing esterases may be located in the soluble fraction of biological systems.

Table 4 shows the effect of *p*H, temperature and substrate concentration on optimization of enzyme activity from both LBB-R strains of *C. septempunctata.* In line with normal expectation for thermolability of an enzyme, the activity profile of both strains was parabolic, rising to a peak at a temperature of 50 °C, followed by a subsequent decline. In *A. gossypii,* an optimal temperature of 40 °C was observed (Owusu *et al.,* 1994). In organophosphate susceptible and resistant strains of *M. persicae,* an optimal temperature of 35 °C has been reported (Sudderuddin, 1973). Also, Zhu & Brindley (1990) working on esterases of *Lygus hesperus* Knight (Hemiptera: Miridae) found the optimum temperature range to be 40 - 45 °C. Both insect strains showed complete *p*H dependency at all levels of *p*H studied. However, while activity for the resistant strain peaked at *p*H 7.5, that of the susceptible strain peaked at *p*H 6.8. This probably suggests that enzyme from the two strains may have different centres of activities, charge mobilities, and thus behave differently towards inhibitors. Substrate-concentration-dependent activity patterns by enzymes from strains of *C. septempunctata* were similar with optimum

TABLE 4
p*H, temperature, and substrate (2-NaA) concentration optima for LBB-S and LBB-R strains of* C. septempunctata

Property	*Working range*	*Optimum value*	
		LBB-S	*LBB-R*
*p*H	6 ~ 8.5	6.89	7.5
Temperture	10 ~ 60	50	50
Substrate concentration (*M*)	1×10^{-5} ~ 1×10^{-1}	2.5×10^{-3}	2.5×10^{-3}

concentration values being exactly the same.

In all cases of *p*H, temperature and substrate concentration studied, however, enzyme activity of most tolerant (LBB-R) strain was higher than that of most susceptible (LBB-S) strain. The advantages of using resistant strains of a predator to control a particular pest species are enormous but will depend on how different the mechanisms of insecticide tolerance will be. Papers concerning the general mechanisms of insecticide tolerance in *C. septempuncatata* and the use of tolerant strains of this predator beetle to control aphids in the glasshouse are being compiled.

References

CROFT, B. A. (1975) Responses of arthropod natural enemies to insecticides. *Ann. Rev. Entomol.* **20**, 285-335.

DAUTERMAN, W. C. (1983) Role of hydrolases and glutathione S-transferases in insecticide resistance. In *Pest Resistance to Pesticides* (ed. Georghiou, G. P. & Saito, T.), pp. 229-247. New York: Plenum.

DEVONSHIRE, A. L. & MOORES, G. D. (1982) A carboxylesterase with broad substrate specificity causes organophosphorus and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). *Pestic. Biochem. Physiol.* **18**, 235-246.

DEVONSHIRE, A. L. & SAWICKI, R. M. (1979) Insecticide-resistance *Myzus persicae* as an example of evolution by gene duplication. *Nature* **280**, 140-141.

DEVONSHIRE, A. L., (1973) The biochemical mechanisms of resistance to insecticides with special reference to the housefly and aphid, *Myzus persicae*. Science **4**, 521-529.

HAMA, H. & HOSODA, A. (1988) Individual variation of aliesterase activity in field populations of *Aphis gossypii* Glover (Homoptera: Aphididae). *Appl. Entomol. Zool.* **23**, 109 - 122.

HOY, M. A. (1990) Pesticide resistance in arthropod natural enemies: variability and selection responses. In *Pest Resistance in Arthropods* (ed. Roush, R. T. & Tabashnik, B. E.), pp. 203-236. New York: Chapman and Hall.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**, 265-276.

MOTOYAMA, N., KAO, L. R., LIN, P. T. & DAUTERMAN, W.C. (1984) Dual role of esterases in insecticide resistance in green rice leafhopper. *Pestic. Biochem. Physiol.* **21**, 139 - 147.

O'BRIEN, P. J., ABDEL-AAL, Y. A., OTTEA, J. A. & GRAVES, J. B. (1992) Relationship of insecticide resistance to carboxylesterase in *Aphis gossypii* (Homoptera: Aphididae) from mid south cotton. *J. Econ. Entomol.* **85**, 651-657.

OPPENOORTH, F. J. (1984) Biochemistry of insecticide resistance. *Pestic. Biochem. Physiol.* **22**, 187-193.

OWUSU, E. O. (1995) Biochemical Elucidation of Dichlorvos Resistance Mechanisms in Cotton Aphid *Aphis gossypii* Glover (Homoptera: Aphididae). Ph.D. Thesis, United Graduate School of Agricultural Sciences, Ehime University, Ehime, Japan.

OWUSU, E. O. & ADJEI-AFRIYIE, F. (1998) Relationship between permethrin susceptibility and elevated esterase activities in cotton aphid (Homoptera: Aphididae) clones. *Gh. J. Sci.* **31-36**, 95-99.

OWUSU, E. O. & HORIIKE, M. (1997) Some properties of 1-chloro-2-dinitrochlorobenzene linked glutathione S-transferase in dichlorvos resistant and susceptible strains of cotton aphid (Homoptera: Aphididae). *J. agric. Sci.*, Camb. **127**, 469-473.

OWUSU, E. O. HORIIKE, M. & HIRANO, C. (1996a) Polycrylamide gel electrophoretic assessments of esterases in cotton aphid (Homoptera: Aphididae) resistance to dichlorvos. *J. econ. Entomol.* **89**, 302-306.

OWUSU, E. O. HORIIKE, M. & HIRANO, C. (1996b) Inhibition of insecticides by partially purified carboxylesterase from *Aphis gossypii* (Homoptera: Aphididae). *J. econ. Entomol.* **89**, 307-310.

OWUSU, E. O., KOMI, K., HORIIKE, M. & HIRANO, C. (1994) Some properties of carboxylesterase from *Aphis gossypii* Glover (Homoptera: Aphididae). *Appl. Entomol. Zool.* **29**, 47-53.

SUDDERUDDIN, K., I. (1973) An *in vitro* study of esterases hydrolysing non-specific substrates of OP-resistant strain of the green peach aphid, *Myzus persicae* (Sulz.) *Comp. Biochem. Physiol.* **44B**, 1067-1076.

ZHU, K. Y. & BRINDLEY, W. A. (1990) Properties of esterase from *Lygus hesperus* Knight (Hemiptera: Miridae) and the role of esterases in insecticide resistance. *J. econ. Entomol.***83**, 725-732.

Received 17 Jan 97; revised 1 Nov 99.