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RELATIONSHIP BETWEEN PERMETHRIN SUSCEPTIBILITY AND ELEVATED ESTERASE IN CLONES OF COTTON APHID (HOMOPTERA: APHIDIDAE)

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Summary

Activities of some hydrolytic enzymes and their relationship to permethrin (a pyrethroid insecticide) susceptibility in the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), were evaluated *in vitro*. All esterases studied, i.e. acetylcholinesterases, butyrylcholinesterases, carboxylesterases, and p-nitrophenyl acetate hydrolyzing esterases, exhibited to definite trend in relation to 50 per cent lethal concentration (LC_{50}) values of permethrin. Thus, resistance to permethrin in cotton aphid is likely to be expressed through causes other than elevated esterase activities.

Introduction

Problems associated with crop infestation by the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), have been on an increase world wide. The insect exhibits seasonal biological variation and possesses differential rates of reproduction both in open fields and grasshouses (Owusu, Horiike & Milano, 1994a). However, one of the factors limiting effective control of this pest is the increasing trend of insecticide resistance.

Generally, resistance in the cotton aphid is speculated to be expressed through multiple mechanisms rather than cross-resistance from a single enzymatic mechanism (Sun *et al.*, 1987). Organophosphate (dichlorvos) resistance in this aphid has been shown to be highly correlated with carboxylesterase activity both quantitatively and qualitatively (Owusu, Horiike & Milano, 1996). On the other hand, the nature of pyrethroid resistance in this insect has yet to be widely studied and the only information available is based mainly on resistance to primicarb (Furk, Powell & Heyd, 1980). Against this background, a study was conducted to characterize susceptibility to permethrin among clones of the insect, and relate it to activity levels of some specific esterases. This is the first step towards a general objective of elucidating the mechanism of permethrin resistance in the cotton aphid.

Experimental

Aphids

Three laboratory strains bred on eggplant, i.e. susceptible (E-S), dichlorvos selected (E-D-R), as well as permethrin selected (E-P-R), were used as standards. The rest were collected from fields around Nankoku City in Kochi Prefecture, Japan, and a clone each was developed from a single apterous female reared on its original host in the insectary (25 ± 2 °C; 16L:8D). Names were assigned to clones based on the host as well as order and year of collection. For example, the first clone developed on eggplant in 1993 was assigned E-1-93, etc.

Chemicals

All chemicals were of the highest commercial grade. The following chemicals were purchased from Wako Chemical Industries Ltd (Osaka): sodium hydroxide (anhydrous), potassium phosphate monobasic, disodium hydrogen phosphate, 1-naphthol, sodium dodecylsulphate (SDS), eserine sulphate, acetylthiocholine iodide (ASCh), butyrylthiocholine iodide (BSCh), and 5,5'-dithiobis-2-nitrobenzoic (DTNB). Azoic diazo component (FBS), 1-naphthyl acetate (1-NaA), 2-napthyl acetate (2-NaA), *p*-nitrophenyl acetate (*p*-

NpA), and polyethylene glycol (Triton X-100) were purchased from Tokyo Kasei Industrial Company, Tokyo, Japan. Permethrin (20 per cent) under the trade name adion, was received from Sankyo Chemical Company Ltd, Japan.

Toxicity test

One end of a glass tube (30 × 20 mm l/ID) was sealed with a piece of nylon cloth. Aphids were then introduced into the tube and the other end sealed with parafilm to prevent escape. Aphids were made to rest at the bottom end sealed with nylon. That end was then soaked in the various concentrations of the test insecticide (dissolved in water) for 30 s. Water was used as a control. Excess liquid was removed by blotting on a piece of filter paper. Aphids were thereafter picked with a soft camel hair brush and placed in plastic cups containing excised leaves of their respective hosts under 25 2 ± °C of temperature. The number of dead insects was counted after 24 h. Fifty and ninety-five per cent lethal concentrations were calculated after correcting for control mortality by the formula of Abbot (1925).

Enzyme preparations

Individual aphids were homogenized in 0.3 ml of potassium phosphate buffer (*p*H 7.0) using a hole slide glass. The resultant solution was then used as enzyme source for carboxylesterase assay. For cholinesterase and *p*-nitrophenyl acetate hydrolyzing esterase determinations, 50 adult female aphids were homogenized in 1 ml of potassium phosphate buffer containing 0.2 per cent of Triton x-100, and centrifuged for 20 min at 3,000 g. The resultant supernatant fraction was utilized as enzyme source for assay.

Carboxylesterase assay

Carboxylesterase assay was based on the original method developed for housefly esterase (van Asperen, 1960), as adopted for cotton aphid carboxylesterase (Owusu *et al.*, 1994b). A typical reaction mixture consisted of incubating 0.1 ml of enzyme with 0.1 ml of 30 *mM* 1-naphthyl acetate in absolute ethanol for 10 min at 40 °C in 2.8 ml of phosphate buffer (*p*H 7.0). After incubation, 0.5 ml solution mixture of sodium dodecyl sulphate-fast blue salt (SDS-FBS) was added for colour development and read thereafter at 600 *nm* on a spectrophotometer against a control that lacked enzyme.

Cholinesterase assay

Cholinesterase was assayed as described for *Nephotettix cincticeps* (Owusu, 1992). A typical reaction mixture consisted of 1.8 ml of potassium phosphate buffer (*p*H 8.0), 50μ*l* of substrate (ASCh or BSCh), 0.1 ml of 0.01 *M* 5,5'-dithiobis-2-nitrobenzoic (DTNB), and 0.1 ml of enzyme extract. After incubation of enzyme and substrate at 30 °C for 5 min, DTNB was added and allowed for an additional incubation time of 10 min. The reaction was stopped by the addition of 0.1 ml of 1×10^{-3} *M* eserine sulphate. Activity was then measured at 412 *nm* on a spectrophotometer.

p-nitrophenyl acetate hydrolyzing esterase (p-NpE) assay

This enzyme was assayed by measuring the production of *p*-nitrophenol from *p*-nitrophenyl acetate (*p*-NpA). The assay was a modified form of that described by Krisch (1966), and consisted of 2.1 ml of potassium phosphate buffer (*p*H 7.0), 40μ*l* of 0.5 *M* p-NpA in acetone, 0.76 ml of 1 per cent Triton x-100 and 0.1 ml of enzyme solution. The reaction mixture was incubated at 25 ± 2 °C for 10 min and subsequently measured at 405 *nm* on a spectrophotometer against a control that lacked enzyme.

Results and discussion

Results of susceptibility of cotton aphid clones to permethrin are shown in Table 1. Contrary to organophosphate resistance which is linked to the aphid host (Owusu, 1995), permethrin resist ever, variability in resistance ratios and slopes of the probit regressions confirms that migration of susceptible and/or resistant strains of *A. gossypii* from one host or place to the other may be limited and that resistance is selected on a relatively small scale (Owusu, 1995).

Fig. 1 shows the relationship between LC_{50} of permethrin and activity levels of 1- and 2-naphthyl acetate hydrolyzing esterases. Fig. 2 also shows same relationship concerning acetyl- and butyrylcholinesterase, as well as *p*-nitrophenyl acetate hydrolyzing esterases. In all cases, no definite trend of relationship was observed.

Table 2 compares the activity levels of esterases from E-P-R strain with those of E-S and E-D-R strains. The E-P-R strain which was selected for resistance from the original E-S strain, showed no superiority over the E-S strain in esteratic activities despite a 13-fold resistance. This *in vitro* assessment was confirmed by polyacrylimade gel electrophoresis which indicated no qualitative differences between the E-P-R and and E-S strains. The E-D-R strain showed an appreciable level of tolerance to permethrin even though slightly lower than the E-P-R strain. This suggests the possibility of cross-resistance by this strain to permethrin.

Permethrin is a synthetic type 1 pyrethroid with a stable alcohol moiety and an unstable dimethylvinyl group on the acid side, modified by chlorine atoms. Consequently, it is supposed to exhibit hydrolytic degradation at the expense of oxidative and other degradations (Matsumura, 1985). Unfortunately, however, its hydrolytic strength was not manifested in the E-P-R- strain. In the mouse system, the relative importance of an esterase attack, as supposed to an oxidative one, is more pronounced for cypermethrin than for permethrin and that in cis-permethrin, it is only 17.3 per cent as against 93.2 per cent for oxidative (Matsumura, 1985).

In the present study, activity of oxidases could not be detected in any of the strains by the aldrin epoxidation method, showing that oxidases may not be directly involved. However, Sun *et al.* (1987) speculated the involvement of mixed function oxidases in resistance of Chinese cotton aphids. An indefinite relationship between resistance and increase in esterase activity has been observed for pirimicarb resistance of this aphid, and that enzyme patterns of pirimicarb resistant strains showed no host-linked relation (Furk, Powell & Heyd, 1980; Takada & Murakami, 1988). In China, the main cause of pyrethroid resistance in cotton aphid has been attributed to the knock down resistant (Kdr) gene (Han *et al.*, 1992). In the present study, the incoherent nature of the results shows that elevated esterase activities play no significant role in permethrin resistance of cotton aphid. As a result, pyrethroid resistance in this aphid might be due to causes other than elevated esterase activities.

TABLE 1

Response of cotton aphid clones to permethrin

Clone	*n*	*LC_{50}(95% FL)*		*X^2*	*Slope(2±SE)*	*RR*
E -1-93	300	84.63	(75.64 - 94.69)	2.36	6.44 (1.33)	1.00
E -3-93	300	375.95	(329.59 - 428.88)	3.63	4.79 (0.74)	4.44
E -4-93	600	383.49	(347.62 - 423.05)	4.44	7.79 (1.44)	4.53
W-2-93	300	395.32	(352.51 - 419.23)	0.95	1.25 (0.51)	4.67
O -1-93	300	457.00	(425.79 - 489.52)	0.92	2.54 (0.18)	5.40
C -1-93	240	468.55	(430.44 - 510.02)	3.34	8.94 (1.52)	5.54
E -6-93	300	473.84	(432.85 - 518.70)	2.98	7.79 (1.26)	5.60
E -5-93	240	488.20	(444.18 - 536.62)	1.42	7.54 (1.45)	5.77
C -2.93	300	508.85	(484.60 - 534.25)	12.56	9.21 (1.12)	6.01

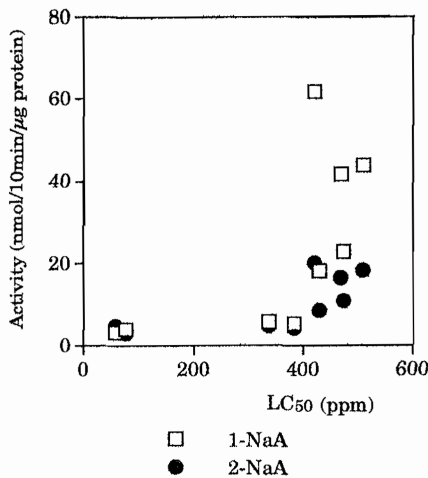

Fig. 1. Relationship between permethrin susceptiblity (LC_{50}) and carboxylesterase activities in cotton aphid clones.

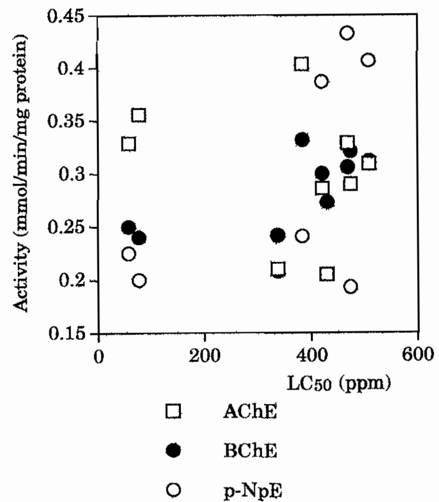

Fig. 2. Relationship between permethrin susceptiblity (LC_{50}) and some esterase activities in cotton aphid clones.

TABLE 2

Relationship between permethrin susceptibility and esterase activites in three laboratory strains of cotton aphid

Strain	LC_{50} (±SE) (ppm)	CarE activity 1-NaA	CarE activity 2-NaA	ChE activity ASCh	ChE activity BSCh	p-NpE activity p-NpA
E-S	58.08 (5.99)	3.32	4.84	0.33	0.25	0.23
E-P-R	746.58 (12.37)	3.21	4.93	0.26	0.24	0.25
E-D-R	658.32 (17.28)	86.27	42.48	0.30	0.29	0.53

CarE activity = *n* mol/10min/µg protein
ChE & *p*-NpE activities = µmol/min/µg protein.

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