Resistance of the Kanzawa Spider Mite to Acaricides with Special Reference to Organophosphorus and Carbamate Compounds

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Resistance of spider mites to acaricides, organophosphates (OPs) in particular, is a world wide problem. Due to intensive cultivation system in Japan, extensive and repeated application of acaricides has been practiced, which resulted in the development of various degrees of resistance in mites. Despite the accumulation of considerable amount of information on acaricide resistance of the fruit tree parasitic mites such as European red mite, *Panonychus ulmi*, and Citrus red mite, *P. citri*, information on tetranychid mite is relatively scanty.

Kanzawa spider mite, *Tetranychus kanzawai*, is recorded in Taiwan, Hongkong, the Philippines and Japan, and is known as a key pest for tea, pear, strawberry, mulberry tree, pulse etc. in Japan.[4]

Susceptibility to acaricides of several strains of the mite collected mainly from strawberry fields was determined by the spray technique (Fig. 1). They showed a high degree of resistance and similar resistance patterns to various OPs. On the other hand, the resistance patterns to specific acaricides varied with populations and acaricides. These results

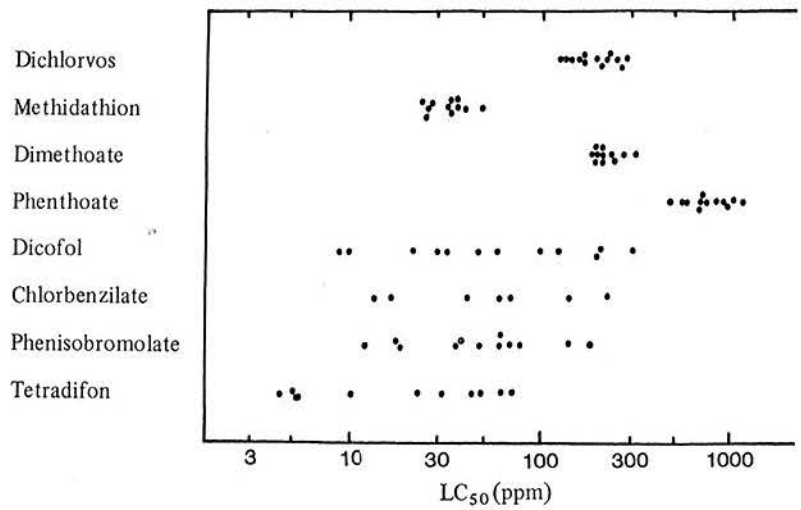

Fig. 1. Susceptibility of the Kanzawa spider mite collected from the field to several acaricides

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show that there is cross resistance to OPs, and imply that major resistance factors are involved in OP resistance, but resistance mechanism to specific acaricides is specific to the compounds.

Mechanism of resistance to OPs and carbamate in the mite

1) Insensitivity of the AChE to OPs and carbamates

To check the relationship between resistance and sensitivity of the AChE from the mite to toxicants, the sensitivity of the AChE from laboratory-selected and field-collected strains of the mite was examined. The bimolecular rate constants k_i for the reaction between various inhibitors and the mite AChE were calculated according to the method of Aldridge (1950).[1] The relation between the resistance factors for the toxicants and relative insensitivity of the AChE to corresponding inhibitors is shown in Fig. 2. Relative insensitivity of the AChE to inhibitors is expressed as the ratio of rate constants; k_i (OP-susceptible strain)/k_i (OP-resistant strain). Generally a good correlation was recognized between *in vivo* resistance to toxicants and *in vitro* insensitivity of the AChE to corresponding inhibitors: the greater the resistance factor is to the inhibitors, the more is the relative insensitivity of the AChE in the homogenate of resistant strains. These results indicate that lowered sensitivity of the AChE is the major mechanism of resistance to these toxicants in the Kanzawa spider mite.[11]

On the other hand, however, no correlation was recognized between the *in vivo* resistance to malathion and *in vitro* sensitivity of the AChE from the resistant strain to malaoxon[5] (Fig. 2). The OP-resistant strains of mite manifested increased esterase and malathion-hydrolyzing activity as compared with those of the susceptible strain,[9] and a correlation between esterase activity and synergism of a mixture of malathion and K-1, a malathion-specific synergist, has been shown.[10] Therefore, these results indicated the possibility of

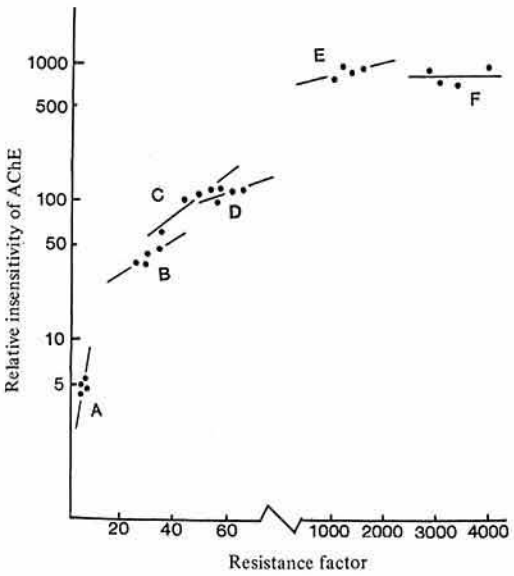

Fig. 2. Relationship between relative insensitivity of the acetylcholinesterase from OP-resistant strains and resistance factors to various inhibitors

detoxification mechanism of resistance to malathion in the mite.

2) Detoxification of the toxicants

The response of four strains of the mite to malathion and the inhibitory effects of phosphorus esters on the *in vitro* degradation of ^{14}C-malathion by the mite homogenates are shown in Table 1. It is obvious from the data that the more resistant the mite is to malathion, the higher is the degradation activity of the homogenate. Compound K-1 exhibited a distinctive inhibitory effect on the *in vitro* degradation of this chemical. Other esters which demonstrated no significant synergism with malathion, however, exhibited no such inhibitory effect on its degradation. These results coincided with the synergistic effect of these compounds with malathion against OP-resistant strains of mite.[8]

In vitro degradation of ^{14}C-malathion by mite esterases from OP-resistant ZoR strain separated by the thin-layer agar gel electrophoretic method, along with the corresponding zymogram for β-naphthyl acetate hydrolysis

Table 1. ***In vitro*** **degradation of malathion with the without synergists by four strains of the Kanzawa spider mite (dpm/2.8 mg mite/30 min)**

Mixture	Strain			
	ZoR	NER	Ns	Nk
^{14}C-malathion	772 (10800)	609 (8200)	172 (32.0)	121 (19.8)
^{14}C-malathion + K-1	290	220	143	128
^{14}C-malathion + K-2	783	602	183	128
^{14}C-malathion + K-9	787	603	188	125
^{14}C-malathion + IBP	766	578	169	119

Figures in parenthesis indicate LC_{50}(ppm) of the strain to malathion.
Synergists concentration: 1×10^{-5}M

is shown in Fig. 3. Six esterase bands were identified, E_1 to E_6, from cathode to anode. Although there was no difference in the relative mobility of these esterase bands among the strains tested, there was qualitative difference in certain bands as judged by the intensity of the color development. Namely, the E_3 and E_4 bands of OP-resistant strain ZoR developed a moderately clear red color, while those of OP-susceptible strains developed a faded red.[9] Thus, the resistance to malathion in the Kanzawa spider mite was determined to be associated with an increased esterase activity at the E_3 and E_4 bands.

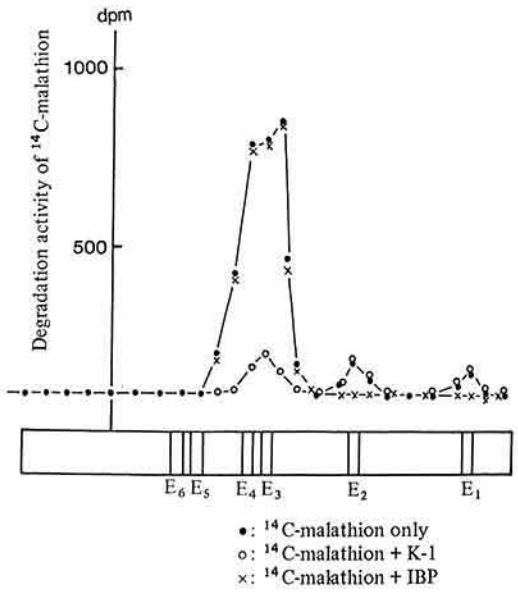

Fig. 3. ***In vitro*** degradation of ^{14}C-malathion by OP-resistant ZoR strain enzymes, and the effect of K-1 and IBP on the activity of esterases separated by agar gel electrophoresis

The malathion degrading activity resolved into three peaks. The major peak position coincided with that of esterases E_3 and E_4, and the two minor positions coincided with that of esterases E_1 and E_2 respectively. The compound K-1, which manifested prominent synergism with malathion to OP-resistant strains,[8] distinctively inhibited the degradation of malathion by E_3 and E_4 bands but did not inhibit its degradation by E_1 and E_2 bands. It was demonstrated that K-1 inhibited the esterase activity of E_3 and E_4 bands but did not inhibit that of E_1 and E_2 bands. On the contrary, IBP did not inhibit the activity of E_3 and E_4 bands. K-2 and TPP did not inhibit the esterase activity of any bands.[9] It is, therefore, likely that the Kanzawa spider mite has esterase active for both β-naphthyl acetate hydrolysis and malathion degradation. It should be noted from these results that in the mite there are at least three malathion degrading esterases which can be classified into two groups for their distinctive response to synergists.

It is difficult to determine whether both responses were catalyzed by the same enzyme or by two different enzymes, since more than two enzymes can have a common electrophoretic mobility. However, the fact that the compound K-1 and IBP selectively inhibited both reactions suggests that the same enzyme is probably responsible for both reactions.

The synergism of K-1 with malathion in

the mite appears to be due to the inhibition of the carboxylesterase which is responsible for the degradation of malathion. The compound, therefore, can be used as a tool for assessing malathion-specific resistance of the mite. The K series of compounds used in this experiment was originally discovered as biological active metabolites of TOCP,[2] and the insecticidal activity and chemical properties of analogous cyclic phosphates have been reported.[5,6] It was demonstrated that K-2 was a potent inhibitor for malathion-carboxylesterase from the housefly, smaller brown planthopper, and the compound manifested a prominent synergism with malathion to these insects.[13] In case of the spider mite, however, K-2 does not manifest synergism with malathion,[8,15] nor, as shown in Table 1 and Fig. 3, does it inhibit the esterases responsible for degradation of malathion. This is in contrast to the compound K-1 which manifested synergism with malathion, and also selectively inhibited the esterase activities (Table 1 and Fig. 3). These results indicate that the properties of the carboxylesterase which hydrolyzes malathion must differ greatly between the insects and spider mites.

Accordingly, the increased esterase activity, at least in part, in the OP-resistant strains of the Kanzawa spider mite is directly connected with the decomposition of malathion, which resulted in partial modification of activity of malathion *in vivo*. A combination of insensitivity of the AChE to inhibitors and the additional mechanism, i.e., an increased activity in degradation of toxicants, might be responsible for the extensive and intensive resistance in spider mite.

The properties of AChE from OP-resistant and susceptible strains of mite

1) Substrate specificity of the AChE

The activity of the AChE of mites susceptible and resistant to OPs in the reaction with acylthiocholine esters is shown in Table 2. The AChE activity of the susceptible strain was higher with propionylthiocholine as a substrate than with acetylthiocholine, but that of the resistant strains was higher with acetylthiocholine than with propionylthiocholine. The activity of the enzyme of both strains with butyrylthiocholine as a substrate was much lower than that with the other two substrates. An almost similar difference between the resistant and susceptible strains was observed in the Michaelis constants (Km) and maximal velocity (Vmax) of the reaction of the enzyme with three acylthiocholine esters (Table 3). Thus, the properties of the AChE from the resistant strains were characterized by lower Vmax and larger Km than those from the susceptible strain in the reaction with propionylthiocholine. These findings can be interpreted as indicating that the AChE of the OP-resistant strain possesses an abnor-

Table 2. Activity of acetylcholinesterase from OP-susceptible (S) and -resistant (R) strains of mites with acylthiocholine esters as substrate

Strain	Substrate				
	ATCh		PrTCh		BuTCh
	2×10^{-4}M	1×10^{-3}M	2×10^{-3}M	1×10^{-3}M	1×10^{-3}M
NsPs (S)	78.9	87.6	88.0	102.4	7.4
NsP (R)	62.7	75.1	26.6	41.6	4.7
ZoR (R)	59.5	68.4	23.4	39.5	3.3

Figures are expressed as μ moles of substrate hydrolyzed by g mites per hr at 30°C.
ATCh: acetylthiocholine, PrTCh: propionylthiocholine,
BuTCh: butyrylthiocholine.

Table 3. Comparisons of maximal velocities (Vmax) and Michaelis constants (Km) of acetylcholinesterases from OP-susceptibles (S) and -resistant (R) strains of the Kanzawa spider mite for acylthiocholines

Strain	Substrate					
	ATCh		PrTCh		BuTCh	
	Vmax	Km × 10^4	Vmax	Km × 10^4	Vmax	Km × 10^4
NsPs (S)	96.9	0.425	109.0	0.418	10.2	3.77
NsM (R)	80.4	0.433	51.3	1.81	8.88	18.1
NsD (R)	82.7	0.489	46.5	1.78	9.20	17.7
NsP (R)	78.9	0.442	50.0	1.75	8.90	18.4
ZoR (R)	72.7	0.451	47.2	2.02	9.16	18.2

ATCh: acetylthiocholine, PrTCh: propionylthiocholine, BuTCh: butyrylthiocholine.
Vmax: Activities are expressed as μ moles acylthiocholines hydrolyzed by g mites per hr at 30°C.

mally weak esteratic site, and that the esteratic site of the enzyme from OP-susceptible strain is wide enough to accommodate propionyl moiety of acylthiocholine esters, while, that of OP-resistant strains is not wide enough to accommodate even a propionyl moiety of acylthiocholine esters.[12]

2) The properties of anionic sites of the AChE

Wilson and Bergman (1950)[16] indicated the presence of two sites, the esteratic and anionic, on the AChE, and cationic part of the substrate is bound to the anionic site on the enzyme not only by ionic but also physical binding forces. With mite AChE no clear pattern of specificity to the N-alkyl substituted acetylcholine was observed, and thus it was conjectured that Van der Waals's binding to the anionic site is more important than coulombic binding for substrate and enzyme conjugation.[3]

Quaternary ammonium is bound to the anionic site on the enzyme and inhibit the AChE activity,[17] so the difference in sensitivity to the compound reflects the changes of anionic site on the enzyme. This compound can, therefore, be used as a tool for assessing the difference of properties of anionic site on the enzyme.

The inhibitory strength of quaternary ammonium compounds on the AChE activity is shown in Table 4. There is no remarkable difference in sensitivity of the AChE from two sources to these compounds, which indicates no evident difference in anionic site on the AChE from two strains of mites. This is consistent with the results on the properties of anionic site on the AChE from the housefly[14] and the green rice leafhopper.[7]

Table 4. The effect of ammonium ion on the activity of acetylcholinesterase from OP-susceptible (S) and -resistant (R) strains of the Kanzawa spider mite

Compound	Inhibition constants (K_i)	
	NsPs(S)	ZoR(R)
Phenyltrimethylammonium	1.3×10^{-4}	1.6×10^{-4}
Tetramethylammonium	4.1×10^{-4}	5.0×10^{-4}

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