(Jour. agric. Res. China) 中華農業研究32(4) : 373~378 (1983)

Insecticide Resistance Study in *Plutella xylostella* (L.)

IV. The Activities of Glutathione-S-transferase in the Organophosphorus-resistant Strains[1]

by

Edward Y. Cheng, Tao-mei Chou and Ching-hua Kao[2]

Abstract : The role of glutathione(GSH)-S-transferase as a detoxication mechanism to organophosphorus insecticides was investigated in the diamondback moth, *Plutella xylostella* (L.). The GSH-S-transferase was prepared fromthe susceptible IL-strain, two organophosphorus-resistant strains and two synthetic pyrethroid-resistant strains. The result indicated that the GSH-S-transferase activities in two organophosphorus-resistant strains were three to four times higher than that of the susceptible IL-strain, i.e., the parental strain from which all resistant strains were derived. Both O-methyl and O-ethyl groups on the organophosphorus insecticides can cause GSH-S-transfer ase activity increases in insecticide pressed diamondback moth population. Hence, the GSH-S-transferase detoxication mechanism is quite specific to the organophosphorus insecticides. The study indicated that the glutathione conjugation is an important resistant mechanism to the organophosphorus insecticides in the diamondback moth.

It has been reported that the increase in metabolism of organophosphorus insecticides by glutathione(GSH)-S-transferase is an important factor for resistance in insects [8]. The O-dealkylation of organophosphorus insecticides by GSH-S-transferase has been well studied in house fly. The quantitative increase in enzyme activity in the resistant strain was responsible for the organophosphorus resistant mechanism [9].

Although this enzyme has been studied in the diamondback moth, *Plutella xylostella*, no conclusion can be made to relate its activity to the organophosphorus resistance [10]. Several pure organophosphorus-resistant diamondback moth strains have been successfully raised in our laboratory and they are very suitable for the GSH-S-transferase study. The result is compared to that of the house fly study to clarify whether or not this organophosphorus resistant mechanism exists in the diamondback moth.

1. Contribution No. 1136 from Taiwan Agricultural Research Institute, the study is supported by NSC research grant 72-0409-B055-03.

2. Senior Entomologist, Project Assistant and Research Assistant respectively, Department of Applied Zoology, TARI. Wufeng, Taichung, Taiwan 431, Republic of China.

Materials and Methods

Test insects

a. susceptible strain : the IL-strain diamondback moth from our 1980-81 collection was used because of its high sensitivity to all insecticides tested[4].

b. resistant strains : four insecticide resistant sub-strains pressed from the IL-strain were used in this study. The sub-strains were separately resistant to profenofos, R. R. (resistant ratio) = 13.0 ; mevinphos, R. R. = 8.3 ; permethrin, R. R. = 6.7 and cypermethrin, R. R. = 4.7.

Enzyme preparations

The enzymes were prepared by homogenizing twenty early 4th instar larvae in a glass homogenizer with 2 ml ice-cold 0.1M Tris-buffer, pH 9.0 for 2 min. The crude homogenate was centrifuged at 3,000g in a refrigerated centrifuge for 10 min. and the supernatant was filtrated through a piece of tissue paper. The filtrated suspension was kept in ice bath and ready for the enzyme activity determination.

Enzyme assay

The GSH-S-transferase activity was determined spectrophotometrically according to Booth's method[1] using 3,4-dichloronitrobenzene as the substrate and three freshly prepared samples were investigated for each diamondback moth strain. The reaction mixture consisted of 0.2 ml enzyme preparation, 0.1mM 3,4-dichloronitrobenzene and 5mM glutathione (reduced form) in 3 ml of 0.1M Tris-buffer, pH 9.0. The reaction was performed at 37°C and the production rate of S-(2-chloro-4-nitrophenyl)glutathione was measured at 344μm by using a Shimadzu spectrophotometer Model UV-210A. The reference cell contained all the components as that of the sample cell except glutathione. Since GSH-S-transferase deteriorated rapidly, the activity had to be determined immediately after the enzyme was prepared. Reduced glutathione was obtained from Sigma Co. and 3,4-dichloronitrobenzene was purchased from Tokyo Kasei Co.

Protein were determined by the Lowry method[7].

Results

In this study, the GSH-S-transferase was detected in all strains of diamondback moth. The native susceptible IL-strain had the lowest activity at 0.017 mM substrate conjugated per mg protein per hour. The problem in the determination of GSH-S-transferase activity was its unstable nature, even though the preparation had been kept in the ice bath. The activity dropped to merely 37% at the end of one and a half hour after the enzyme was prepared and only 5% activity remained after three hours. The deterioration of this enzyme was illustrated in Fig. 1 and adding 5mM EDTA or 0.01% BSA was not able to prevent the deterioration (Table 1).

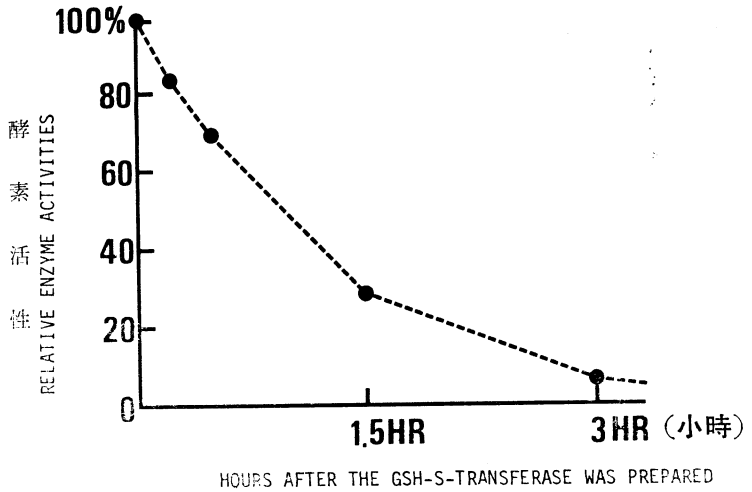


Fig. 1. The deterioration of glutathione-S-transferase activity after the enzyme was prepared.

圖1. GSH-S-transferase活性降低情形

Table 1. The effect of EDTA and BSA in preventing the deterioration of glutathione-S-transferase activity *in vitro*.

表1. 添加 EDTA 及 BSA 保護 GSH-S-transferase 活性試驗

Treatment	Relative activities of enzyme	
	fresh (%)	1 hr after (%)
+EDTA (5mM)	100	38
+BSA (0.01%)	100	47
Check	100	36

EDTA : ethylenediamine tetraacetate

BSA : bovine serum albumin

In Table 2, the activities of GSH-S-transferase of the susceptible IL-strain, organophosphorus resistant sub-strains and synthetic pyrethroid resistant sub-strains were compared. Along with the enzyme activities, the resistant ratio of each resistant strain to its correspondent insecticide was also presented. Table 2 listed the result of the changes in GSH-S-transferase activity in relation to that of the susceptible IL-strain. The activity increased 3.5 folds in both organophosphorus resistant sub-strains. However, no significant change in enzyme activity can be observed in two synthetic pyrethroid resistant sub-strains. Also, only some minor increases can be detected in the cypermethrin resistant sub-strain.

Table 2. Comparison of glutathione-S-transferase activities in susceptible, organophosphorus-resistant and synthetic pyrethroids-resistant diamondback moths
表2. 各品系小菜蛾之GSH-S-transferase活性比較

Strain	R. R.[a]	Enzyme activity[b]	E_r/E_s[c]
Susceptible IL-	—	0.017	—
Profenofos-resistant	13.0	0.056	3.34
Mevinphos-resistant	8.3	0.038	3.37
Permethrin-resistant	6.7	0.020	1.18
Cypermethrin-resistant	4.7	0.028	1.66

a. R. R. : resistant ratio
b. mM substrate conjugated/mg protein • hr
c. E_r : enzyme activity of resistant strain
E_s : enzyme activity of susceptible strain

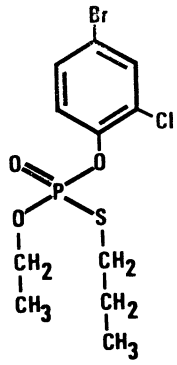
Disussion

The diamondback moth, *P. xylostella*, is an insect which can develope insecticide resistance rapidly as reported by Chou and Cheng [5]. Following the long history of applying organophosphorus insecticides to control the agricultural pests in Taiwan, the diamondback moth has already gained certain degree of resistance [2,3,4,6]. Even the susceptible IL-strain has shown some signs of organophosphorus resistance [5]. However, the IL-strain can still respond to further organophosphorus insecticides press in our laboratory with an increase in resistance which magnituded 8.3 for mevinphos and 13.0 for profenofos. Coincidently, both laboratory induced organophosphorus resistant sub-strains have shown significant increase in GSH-S-transferase activities.

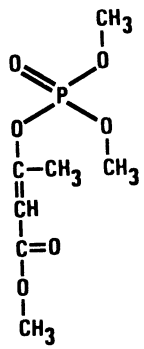
The GSH-S-transferase activities in two synthetic pyrethroid sub-strains remained the same as that of the susceptible IL-strain, i.e., the parental strain. Obviously, the selection against synthetic pyrethroids has not resulted in higher GSH-S-transferase activities in the diamondback moth populations.

The responses of GSH-S-transferase in different sub-strains of the diamondback moth were coincident with the observation of that in the house fly, a marked increase in enzyme activity found to be parallel to the organophosphorus resistant level [9]. Since no significant increases in this enzyme were detected in synthetic pyrethroids resistant sub-strains, this mechanism of resistance appears to be unique in insects resistant to organophosphorus insecticides. The increases of this enzyme can be attributed to more detoxication of organophosphorus insecticides *in vivo* by O-dealkylation. The similar response of GSH-S-transferase in the diamondback moth to profenofos and mevinphos may indicate that both demethylation and desethylation or other dealkylation reactions can happen in the diamondback moth. The result of this study indicated that the

GSH-S-transferase is one of the organophosphorus resistant mechanisms in the diamondback moth as well as that reported in the house fly.



Profenofos



Mevinphos

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小菜蛾抗藥性之研究

IV. Glutathione-S-transferase 在抗有機磷品系中之變化[1]

鄭 允 周桃美 高靜華[2]

利用在宜蘭所採得之感性品系小菜蛾，在室內誘發抗佈飛松及抗美文松兩有機磷劑品系，再行比較該等品系小菜蛾體內解毒酵素 GSH-S-transferase 活性之變化。試驗中利用抗百滅靈及抗賽滅寧二品系作對照，結果發現該解毒酵素的確在抗有機磷品系中提高約四倍，幾乎相當於該品系實際增加之抗性指數(Resistant Ratio)，而抗合成除蟲菊精之品系均無此酵素活性提高之現象，顯然 GSH-S-transferase 係一抗有機磷之專有機制，此結果與國外報導 GSH-S-transferase 爲家蠅抗有機磷之主要機制結果相符，因此證明此一抗藥機制亦存在於小菜蛾體內。

試驗中並發現，小菜蛾體內之 GSH-S-transferase一經分離出體外，活性就開始下降，添加 EDTA及BSA均不能阻止活性下降之現象及程度，三小時內活性下降接近零點。

1.臺灣省農業試驗所 研究報告 第 1136 號。本計畫承國科會補助（編號72-0409-B055-03），謹致謝忱。
2.本所應用動物系研究員、計畫助理及助理。臺灣省 臺中縣 霧峰鄉。