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

## VI. An Experimental Analysis of Organophosphorus and Synthetic Pyrethroid Resistances[1]

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

### Abstract

The organophosphorus and synthetic pyrethroid resistances in diamondback moth, *Plutella xylostella* (L.), were compared in two groups of resistant strains. The first group consisted of seven field strains which carried mixed origins of resistance; the second group were six laboratory-bred strains specially selected by designated insecticides so that the insects with known origins of resistance. The resistance and cross resistance characters of laboratory-bred strains are used in analyzing and explaining the test results of field strains.

The resistance inducing ability and the cross resistance relationship of organophosphorus and synthetic pyrethroids were the main target of this investigation. Variable responses in diamondback moth were obtained for different insecticides. There are fast inducers for resistance as profenofos and fenvalerate; compounds induced resistance slowly as mevinphos, prothiofos, permethrin and cypermethrin. Stability of the resistance was also noticed as both mevinphos and prothiofos induced unstable resistance. Generally, the results of laboratory simulation in regard of organophosphorus were similar to what were actually happened in field strains; on the other hand, the laboratory-bred synthetic pyrethroid resistant strains failed to attain the high resistant level of those field strains.

In cross resistance tests, the organophosphorus insecticides caused significant cross effect on many other organophosphorus compounds with few exceptions. The cross resistance among synthetic pyrethroids were rather common except the diamondback moth responded much stronger to fenvalerate and decamethrin than permethrin and cypermethrin. The resistance crossed over from organophosphorus to synthetic pyrethroids was evident in our laboratory-bred strains while the synthetic pyrethroid resistance crossed to organophosphorus was rather minor and selective. From the evidence of cross resistance, the high synthetic pyrethroid resistance of field diamondback moth may result from the combined effects of many insecticides.

In a cross examination of permethrin- and cypermethrin-resistant strains for the

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[2] Senior entomologist, project assistant and research assistant respectively, Department of Applied Zoology, TARI, Wufeng, Taichung, Taiwan 431, Republic of China.

possible role of extra alpha-cyano group on some synthetic pyrethroids, the result indicated the diamondback moth responded similarly to both compounds in resistance as the presence of cyano group will not discriminate the resistance mechanism.

## Introduction

In Taiwan, the organophosphorus and synthetic pyrethroids are the most-used insecticides for the control of diamondback moth, *Plutella xylostella* (7), and most of the field populations of this pest have developed resistance (1,5,6). The resistance problem not only intensified the spraying schedule but also rendered vegetable farmers constantly searching for a better insecticide. Taiwan Agricultural Research Institute had initiated a program in this matter which included both the survey and the research in hope to understand the causes and work out proper solutions.

The survey conducted in 1980 indicated that the west coast DBM populations on this island were proned to resistance (2). Only in I-lan area, a northeastern county, still harbored a susceptible strain. The I-lan strain was subsequently cultured in TARI as the native susceptible strain for the resistance investigation.

By using the I-lan strain as the parents, strains resistant to different insecticides were obtained. This laboratory simulation study would reveal the resistance induction ability of a tested insecticide and allowed the researchers to examine cross resistance characters of different strains. The test results on organophosphorus compounds and synthetic pyrethroids are reported here.

## Materials and methods

**Insecticides :**

All insecticides used were commercially formulated products. Insecticides used for the resistance induction were profenofos, prothiofos, mevinphcs, permethrin, cypermethrin and fenvalerate. Other insecticides included in both the susceptibility and cross resistance tests were prophos, cyanophenphos, methidathion, mephosfolan, phenthoate, diethquinalphion, decamethrin, flucythrinate, cartap and carbofuran. Insecticides were diluted in distilled water to proper concentrations and sprayed through the Burkard Potter spray tower.

**Insect materials :**

Susceptible strains : Two DBM samples were collected from the same I-lan area, where harbors a susceptible population, except the collecting dates were three years apart.

Field resistant strains : Seven DBM samples were collected in 1984 from Chia-li, Feng-shan, Hua-lien, Lu-chu, Ma-tou, Ping-tung and Ta-hu. Those were the areas with known resistance problems.

Laboratory-bred resistant strains : Six resistant strains were separately selected by profenofos, prothiofos, mevinphos, permethrin, cypermethrin and fenvalerate for more than 10 generations. Usually, one thousand third instar larvae were treated with the selected insecticide at the dosage of $LC_{75}$ and the survivors were subsequently collected for propagating the next generation. A new $LC_{75}$ was re-determined for each generation on 100-200 early emerged larvae before the actual press.

**Testing procedures :**

The susceptibility test for resistance and cross resistance was carried out on every DBM strains. Seven dosages of an insecticide within the range of $LC_{10}$ and $LC_{95}$ were made in distilled water and each dosage was tested on forty to sixty third instar larvae. Results of all tests were analyzed in probits.

Newly obtained $LC_{50}$ of various insecticides of a resistant strain were compared to that of the susceptible I-lan ('80) strain to detect the occurrence and extent of resistance and cross resistance.

The testing method is the same as the usual spraying method adapted in this laboratory(4). The mortality of treated insects was counted 24 hours after the spray; the post treatment holding temperature and relative humidity were controlled at 25±1°C and 85±10% respectively.

## Results

The ability of diamondback moth in developing resistance toward insecticides has been reported before(3,4) and is demonstrated again in this study. The results of resistance induction study were summarized in Table 1. A minimum of ten or more generations of selection by insecticides was proceeded before we determining the final characters of resulted resistant strains. The DBM responded to insecticides differently as the magnitude of resistance varied. Profenofos and fenvalerate received the strongest response in organophosphorus and synthetic pyrethroids respectively. More gentle responses were observed for prothiofos, mevinphos, permethrin and cypermethrin, and the resistance ratios ranged from five to ten. During the selecting processes, we also noticed that both prothiofos and mevinphos induced unstable resistances which fluctuated constantly and made the selection difficult. In Table 2, susceptibilities of both field resistant and susceptible

**Table 1.** The characters of strains specially selected by different insecticides from a native susceptible DBM strain*

| Insecticides | No. of generations | Resulted resistance | $LC_{75}$ (PPM) | |
|---|---|---|---|---|
| | | | original | after press |
| Profenofos (OP) | 12 | 31.60x | 230 | 7,290 |
| Prothiofos (OP) | 14 | 4.56x | 560 | 2,555 |
| Mevinphos (OP) | 20 | 8.00x | 180 | 1,440 |
| Permethrin (Syn. pyr.) | 11 | 6.30x | 28 | 176 |
| Cypermethrin (Syn. pyr.) | 11 | 4.70x | 53 | 249 |
| Fenvalerate (Syn. pyr.) | 12 | 14.34x | 23 | 330 |

*The susceptible I-lan ('80) strain

**Table 2.** Regional variation of DBM susceptibilities to various insecticides in Taiwan (LC$_{50}$, in ppm) .

| Insecticides* (ppm) | IL ('80) | IL ('83) | HL | PT | MT | CL | TH | LC | FS** |
|---|---|---|---|---|---|---|---|---|---|
| Mevinphos | 70 | 100 | 500 | 320 | 360 | 280 | 300 | 270 | 420 |
| Profenofos | 100 | 120 | 1,010 | 580 | 960 | 860 | 1,200 | 1,450 | 680 |
| Prophos | 170 | 320 | 810 | 1,130 | 1,300 | 420 | 830 | 850 | 3,040 |
| Cyanophenphos | 190 | 100 | 770 | 1,320 | 1,160 | 1,430 | 2,600 | 1,670 | 3,360 |
| Methidathion | 270 | 560 | 1,030 | 1,010 | 1,380 | 790 | 990 | 1,300 | 640 |
| Prothiofos | 310 | 420 | 2,010 | 2,830 | 2,530 | 1,500 | 2,530 | 3,120 | 1,250 |
| Mephosfolan | 360 | 370 | 790 | 1,070 | 1,920 | 940 | 1,310 | 1,270 | 1,570 |
| Phenthoate | 670 | 280 | 630 | 770 | 1,920 | 890 | 960 | 4,200 | 1,520 |
| Diethquinalphion | 230 | 440 | — | — | — | — | 8,140 | 9,010 | — |
| Decamethrin | 4 | 12 | 130 | 50 | 290 | 160 | 270 | 340 | 600 |
| Fenvalerate | 9 | 41 | 430 | 90 | 820 | 510 | 1,260 | 830 | 780 |
| Permethrin | 14 | 16 | 220 | 50 | 220 | 140 | 440 | 530 | 740 |
| Cypermethrin | 19 | 41 | 220 | 100 | 260 | 150 | 270 | 250 | 370 |
| Cartap | 290 | 660 | 860 | 900 | 920 | 830 | 1,150 | 510 | 480 |
| Carbofuran | 120 | 150 | 680 | 820 | 640 | 690 | 720 | 1,410 | 1,020 |

*profenofos: selecron; prophos: mocap; cyanophenphos: surecide; methidathion: supracide; prothiofos:tokuthion; mephosfolan: cytrolane; phenthoate:elsan; diethquinalphion:bayrusil.

**IL : I-lan, HL : Hua-lian, PT : Ping-tung, MT : Ma-tou, CL : Chia-li, TH : Ta-hu, LC : Lu-chu, FS : Feng-shan. The resistant population were sampled in 1983-84.

DBM in regard to fifteen commonly used insecticides were reported. The results confirmed that I-lan area still harbored the same susceptible population after a period of three years from 1980, although signs of synthetic pyrethroid resistance appeared. In contrast, all seven samples from west coast showed distinct resistances as their insensitivities to almost all tested insecticides. The information was further converted into resistant ratios in Table 3. On the aspect of magnitude, the resistance problem of synthetic pyrethroid is definitely greater than that of organophosphorus insecticide.

The cross resistance of both laboratory-bred and field-collected resistant DBM strains were compared in Table 4 and 5. Profenofos and prothiofos were succeeded in transforming the susceptible strain into the resistant strains, which were strongly resemble to the organophosphorus resistance of west coast strains. On the contrary, mevinphos failed of doing the same and demonstrated that it is not a strong resistance inducer.

**Table 3**. Regional variation of DBM resistances* to various insecticides in Taiwan (in resistance ratios)

| Insecticides | IL ('80) | IL ('83) | HL | PT | MT | CL | TH | LC | FS** |
|---|---|---|---|---|---|---|---|---|---|
| Mevinphos | 1.0 | 1.4 | 6.9 | 4.4 | 4.9 | 3.8 | 4.1 | 3.7 | 5.7 |
| Profenofos | 1.0 | 1.2 | 10.1 | 5.8 | 9.6 | 8.6 | 12.0 | 14.5 | 6.8 |
| Prophos | 1.0 | 1.9 | 4.7 | 6.7 | 7.6 | 2.4 | 4.9 | 5.0 | 17.9 |
| Cyanophenphos | 1.0 | 0.5 | 4.1 | 6.9 | 6.1 | 7.5 | 13.7 | 8.8 | 17.7 |
| Methidathion | 1.0 | 2.1 | 6.9 | 4.3 | 4.9 | 3.8 | 4.1 | 3.7 | 2.4 |
| Prothiofos | 1.0 | 1.3 | 6.5 | 9.1 | 8.2 | 4.8 | 8.2 | 10.1 | 4.0 |
| Mephosfolan | 1.0 | 1.0 | 2.2 | 3.0 | 5.3 | 2.6 | 3.6 | 3.5 | 4.3 |
| Phenthoate | 1.0 | 0.4 | 0.9 | 1.1 | 2.9 | 1.3 | 1.4 | 6.1 | 2.3 |
| Diethquinalphion | 1.0 | 1.9 | — | — | — | — | 35.4 | 39.2 | — |
| Decamethrin | 1.0 | 3.2 | 35.5 | 14.5 | 80.1 | 45.6 | 73.8 | 93.5 | 165.6 |
| Fenvalerate | 1.0 | 4.9 | 52.1 | 10.9 | 99.2 | 61.5 | 152.0 | 99.6 | 93.9 |
| Permethrin | 1.0 | 1.1 | 15.9 | 3.8 | 15.5 | 10.1 | 31.1 | 37.8 | 52.8 |
| Cypermethrin | 1.0 | 2.2 | 11.8 | 5.0 | 13.9 | 8.1 | 14.4 | 13.3 | 19.4 |
| Cartap | 1.0 | 2.3 | 3.0 | 3.1 | 3.2 | 2.9 | 4.0 | 1.8 | 1.7 |
| Carbofuran | 1.0 | 0.6 | 5.6 | 6.9 | 5.3 | 5.7 | 5.9 | 11.7 | 8.5 |

*relative to susceptible strain-IL ('80)

**IL : I-lan, HL : Hua-lien, PT : Ping-tung, MT : Ma-tou, CL : Chia-li TH : Ta-hu, LC : Lu-chu, FS : Feng-shan.

**Table 4**. The susceptibilities of different diamondback moth strains to various organophosphorus compounds ($LC_{50}$, in ppm)

| Insecticides | Susceptible strains | 7 wild strains | OP-pressed strains | | | Syn. Pyr.-pressed strains | | |
|---|---|---|---|---|---|---|---|---|
| | | | Prof. | Prot. | Mev. | Fev. | Per. | Cyp. |
| Mevinphos | 70-100 | 270- 500 | 334 | 326 | 409 | 459 | 276 | 253 |
| Diethquinalphion | 230-440 | 8,140-9,010 | 8,021 | 11,366 | 1,290 | 254 | 694 | — |
| Cyanophenphos | 100-190 | 770-3,360 | 3,825 | 2,200 | 686 | 551 | 1,881 | — |
| Phenthoate | 280-670 | 630-4,200 | 2,140 | 337 | 590 | 1,574 | 416 | — |
| Prophos | 170-320 | 420-3,040 | 1,182 | 763 | 826 | 537 | 354 | — |
| Methidathion | 270-560 | 640-1,380 | 600 | 425 | — | 689 | 1,044 | 980 |
| Mephosfolan | 360-370 | 790-1,920 | 1,313 | 1,560 | 608 | 395 | 432 | — |
| Profenofos | 100-120 | 580-1,450 | 3,330 | 1,938 | 444 | 225 | 753 | 957 |
| Prothiofos | 310-420 | 1,250-3,120 | 1,124 | 1,130 | 643 | 745 | 1,387 | — |

Although the cross resistance among organophosphorus insecticides are common and closely related, there are some exceptions. For examples, the prothiofos-resistance was crossed to phenthoate and methidathion; the profenofos-resistance was not crossed to methidathion.

Three synthetic pyrethroid selected strains also became less sensitive to organophosphorus compounds. The presence of cross resistance is evident except the extent is not comparable to that of profenofos and prothiofos.

For similar numbers of pressed generations, the selection failed to raise the synthetic pyrethroid resistance to the degree that was observed in the field strains and louger selection may be necessary to attain higher resistance. We did not ignore the possibility of cross resistance from certain organophosphorus insecticides, since high synthetic pyrethroid resistance has been confirmed in both prothiofos- and profenofos-resistant DBM in this study (Table 5).

**Table 5.** The susceptibilities of different diamondback moth strains to five synthetic pyrethroids ($LC_{50}$, in ppm)

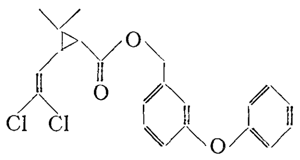
| Strains | Permethrin | Cypermethrin | Fenvalerate | Decamethrin | Flucythrinate |
|---|---|---|---|---|---|
| Susceptible strains | 14-16 | 19-41 | 9-41 | 4-12 | 29-41 |
| 7 wild strains | 50-740 | 100-370 | 90-1,260 | 50-600 | 100-800 |
| Permethrin-resistant strain | 101 | 138 | 240 | 52 | 104 |
| Cypermethrin-resistant strain | 77 | 160 | 340 | 81 | 115 |
| Fenvalerate-resistant strain | 60 | 115 | 136 | 49 | — |
| Profenofos-resistant strain | 71 | 89 | 90 | 51 | — |
| Prothiofos-resistant strain | 327 | 156 | 67 | 88 | — |
| Mevinphos-resistant strain | 38 | 31 | 15 | 13 | — |

The cross examination between permethrin and cypermethrin in resistant strains was aimed to explore the possible action of alpha-cyano group of some synthetic pyrethroids. The test result in Table 6 indicated that this functional group makes no difference in the DBM resistance.

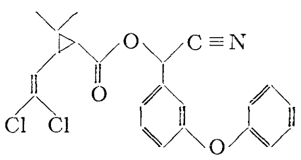
**Table 6.** The cross test of two synthetic pyrethroid-resistant DBM strains to permethrin and cypermethrin*

| Strains | Permethrin-$LC_{50}$ (R. R. **) | Cypermethrin-$LC_{50}$ (R. R. ) |
|---|---|---|
| I-lan | 14 ppm (1.0x) | 19 ppm (1.0x) |
| Permethrin-selected | 101 ppm (7.2x) | 138 ppm (7.3x) |
| Cypermethrin-selected | 77 ppm (5.5x) | 160 ppm (8.4x) |

*Permethrin



Cypermethrin



**R. R. : resistance ratio

## Discussion

The resistance responses of DBM in the organophosphorus insecticides selection study matched closely with what were observed in the field resistant strains. The laboratory-selected organophosphorus resistant DBM also developed cross resistance to most of other organophosphorus compounds. For instance, profenofos-selected strain became resistant to diethquinalphion, cyanophenphos and phenthoate; prothiofos-selected strain was cross resistant to diethquinalphion, cyanophenphos and mephosfolan.

Comparatively, the most-used mevinphos was a compound with the least effect in inducing both resistance and cross resistance as had been reported earlier(4). In general, DBM was not able to generate high resistance toward mevinphos either in field or in laboratory. The fact was further confirmed in profenofos- and prothiofos-selected strains, which were not seriously cross resistant to mevinphos despite their high cross resistance to several other organophosphorus insecticides.

After selected by organophosphorus insecticides, the I-lan strain behaved exactly the same as the west coast strains in its resistance characteristics, which points out that the I-lan strain is not’ physiologically different from the DBM of west coast. It also becomes clear that some organophosphorus compounds will not induce cross resistance to other organophosphorus compounds and this kind of information is very useful in planning the insecticide alternation for the control of DBM.

In the aspect of synthetic pyrethroid resistance, we have found that the result of laboratory simulation was not quite similar to what was happened in the field. The resistance of three laboratory-bred strains could not attain the high resistance level of field strains. Since some organophosphorus insecticides could result cross resistance in synthetic pyrethroids, it is possible that the high resistance found in field was a combined effect of many insecticides.

In field survey, the DBM were frequently more resistant to fenvalerate and decamethrin than to permethrin and cypermethrin(4,6). Similar tendency was confirmed in the laboratory as fenvalerate had a very high resistance induction effect, while permethrin and cypermethrin only had moderate resistance induction rates. This fact makes the positive identification of a synthetic pyrethroid resistant strain easier, since the susceptible DBM is more sensitive to decamethrin and fenvalerate than permethrin and cypermethrin. Whenever this relation is reversed in a particular DBM strain will be a good indication of the presence of synthetic pyrethroid resistance. In the cross examination of permethrin- and cypermethrin-selected strains by permethrin and cypermethrin, the results showed that the responses of both strains were similar and the extra alpha-cyano group on cypermethrin molecule did not cause different resistance. Hence, we believe that this side group is not involved in the resistance mechanism of DBM.

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# 小菜蛾抗藥性之研究

## Ⅵ.有機磷及合成除蟲菊精抗性試驗及綜合分析[1]

鄭 允 周桃美 高靜華[2]

### 摘要

有機磷及合成除蟲菊精類殺蟲劑爲本省蔬菜常用藥劑，亦爲小菜蛾之主要防治藥劑。由於小菜蛾已對此二類化合物產生高度抗藥能力，常導致防治困難及用藥過量之殘毒問題，此研究係對此二類主要防治用藥之效能加以澄淸，並對小菜蛾抗藥反應之特質進行深入瞭解，期能在化學防治之策略上運用所得資料，提供有效的改進措施。爲求愼重起見，在試驗材料上選取兩類抗性起源不同的小菜蛾，測定其對不同藥劑之抗藥性及交互抗性，以爲分析及解釋之基礎。第一類係田間材料，七個樣品均採自抗藥地區；第二類材料係室內培養之抗性品系，以宜蘭的感性小菜蛾爲蟲源，分別利用佈飛松、普硫松、美文松三種有機磷劑，及芬化利、百滅寧及賽滅寧三種合成除蟲菊精劑，進行汰選，汰選標準爲 $LC_{75}$，經 10～20 代，汰選出具已知抗性起源之品系。以此二類試驗材料，一爲混合抗藥起源之族羣，另一爲已知其抗藥起源之族羣，共同進行對有機磷及合成除蟲菊精類殺蟲劑之抗性測定，並檢討其間之交互抗性關係，後一類材料所得結果有助於解釋具多重抗藥起源的田間族羣所表現之特性。測定結果發現：（1）本省小菜蛾就抗藥性而言，各地蟲系的原始起源可能均相同。因爲目前仍屬感性的宜蘭品系，在室內經過有機磷汰選後，所表現抗藥程度及特質即與西岸各地之抗藥品系小菜蛾相似。（2）有機磷劑間之交互抗性一般甚爲明顯，但亦有某些藥劑之間，並無交互抗性關係存在。（3）有機磷之抗性亦能對合成除蟲菊精劑產生交互抗性。（4）合成除蟲菊精類藥劑間之交互抗性具共通性，且小菜蛾對芬化利和第滅寧之反應較對百滅寧及賽滅寧爲迅速，所得抗藥性亦較高。此類藥劑亦能引起小菜蛾對少數有機磷產生交互抗性。（5）就抗藥性之誘發而言，無論有機磷或合成除蟲菊精類殺蟲劑，均有强弱之分。如佈飛松，芬化利即有强烈誘發抗藥性之能力；而普硫松、美文松、百滅寧及賽滅寧誘發抗性的能力明顯地較弱。此外，美文松及普硫松誘發之抗藥性較不穩定。（6）合成除蟲菊精劑中，有的分子結構中含 $\alpha$-cyano 基，有的則無，以百滅寧及賽滅寧進行此一問題之交互檢定，（因二藥劑分子構造類似，僅賽滅寧多含 $\alpha$-cyano 基）所測結果幾乎完全相同，顯然此一分子構造上的差別與小菜蛾的抗藥機制無關。

由以上結果，我們已可逐漸選擇誘發抗性速度較緩，且交互抗性較單純的殺蟲劑作爲防治藥劑；並可在抗藥性產生後，選擇無交互抗性關係的藥劑取代之。

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[1] 臺灣省農業試驗所 研究報告第 1204 號。本計畫承國科會補助（編號73-0409-B055--05），簡淑貝、盧燕鈴、林美玲協助工作，謹此一併致謝。

[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。