

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

### VII. Action Patterns of Insecticides and the Resistance Impacts

Ching-hua Kao, Edward Y. Cheng and Doun-fang Lin

**Abstract:** Both the initial and residual activities of 13 organophosphorus insecticides, 5 synthetic pyrethroids, 2 carbamates and 2 tertiary amines on the diamondback moth, *Plutella xylostella* (L.) were determined. All the synthetic pyrethroids exhibited good initial activities but followed with poor residual effects. Two tertiary amines and carbofuran have similar residual activities except the strengths were different. Methomyl showed no initial action as well as residual effect. The organophosphorus insecticides were the largest chemical group subjected to the test and they demonstrated various action patterns. Some of them showed high initial and residual activities but others were not.

The impacts of insecticide resistance on the effect of insecticides were also measured. Once the insecticide efficacy was affected by resistance, the activity profile was lowered and shortened. The prothiophos-selected resistant DBM strain was used to check the validity of the testing method and the result was satisfactory as the test indeed pinpoint the prothiophos-resistance is the origin of resistance. This testing method was also carried out on the field-collected DBMs from different geographic origins to clarify their insecticide resistance characteristics.

### Introduction

Up to 1985, more than thirty insecticides were officially registered for diamondback moth, *Plutella xylostella* (L.), control in Taiwan<sup>(7)</sup>. Although these insecticides belong to a variety of chemical groups, recommended application methods for their usages were rather similar. Does that mean they have similar actions on the DBM? Surprisingly, no detail on the actions of different insecticides had ever been reported. During

- 
1. Contribution No. 1265 from Taiwan Agricultural Research Institute. This study was supported by Council for Agricultural Planning and Development grants 74-4.1-28(05), 74-4.1-147(03) and grant from National Science Council NSC-74-0409-B055-02.
  2. Research assistant, senior entomologist and project assistant respectively, Department of Applied Zoology, TARI, Wufeng, Taichung, Taiwan 431, ROC.
  3. Thanks due to Ms Su-bay Jean and Ms Yen-ling Lu for their supporting works.

our investigation into insecticide resistance in DBM, many testing methods were adapted on the presumption that all insecticides act in the same way. The 24 hours post-treatment count of mortality is one example which presumes that all insecticides exert their maximum effects within that pre-set period<sup>(1)</sup>. Some insecticides were known for their prolonged or residual activities and would be misjudged in the case. If we try to resolve the complicated resistance phenomenon in the DBM, the knowledge of action patterns of different insecticides is definitely essential to the researchers.

In this investigation, we have studied two gross aspects of an insecticide i.e., the initial and the residual effects. DBMs with different resistance backgrounds were compared in the study in order to verify the validity of test results and to explore the possibility of using the same testing method to screen the effective insecticides for field control. The measure is thus needed for practical purpose, as we have noticed in our previous studies that some insecticides still provide satisfactory control for the field DBM, despite their long-standing usages in Taiwan<sup>(5)</sup>.

### **Materials and methods:**

1. Insecticides: The names, formulations and dilutions of tested insecticides were adapted from the Plant Protection Manual<sup>(7)</sup> and were listed in Table 1. Few registered insecticides were omitted due to the overlapping registration or well-known resistance problems.

2. Insect strains: The native IL-strain of DBM<sup>(1)</sup> was chosen as the susceptible standard in the insecticide action determination. The resistant LC-strain<sup>(1)</sup> was used to test the impact of resistance. A laboratory prothiophos-selected strain (R.R.=5.0) served as the known standard resistant strain<sup>(3)</sup>. Field DBMs were collected from two separate areas and they are 2 samples from Matsu isles representing the low resistance group, and 4 samples from the northwest of Taiwan representing the high resistance group. The presumed resistance magnitudes were suggested from the intensity of insecticide usage.

3. Testing procedures: The common cabbage seedlings of 10-15 leaves stage were planted in pots and arranged in rows for the testing sprays in greenhouse. Commercial grade insecticides were diluted in tap water and applied through a hand-operated tank sprayer. After the treatment, 20-30 third instar DBM larvae were transferred to the wet leaf surface and contacted thoroughly with insecticide before the leaf was cut from the plant. Treated leaf and insects were then transferred into a petri dish and held in a 25°C constant temperature cabinet for the mortality counts; all observations were made in three replicates. Treated insects were continually fed with leaves taken from the same treated plant, and the mortality before pupation was summed as the control efficiency of the day sampled. For the residual effect determination, fresh third instar DBM larvae were transferred onto the leaves taken from treated plants in the following days, and the testing procedure was the same as that of the initial effect determination i.e., the cumulate mortality before pupation was counted as the final result.

**Table 1.** The names, formulations and dilutions of tested insecticides

Names	Formulations	Dilutions
Pirimiphos-methyl	26.3% E. C.	500×
Diethquinalphion	25.0% E. C.	500×
Mephosfolan	25.0% E. C.	300×
Dichlorvos	50.0% E. C.	1,000×
Diazinon	60.0% E. C.	1,500×
Naled	58.0% E. C.	1,000×
Phenthoate	50.0% E. C.	1,000×
Mevinphos	25.3% E. C.	500×
Pyridaphenthion	40.0% E. C.	800×
Acephate	25.0% E. C.	800×
Parathion*	47.0% E. C.	1,000×
Salithion	25.0% E. C.	500×
Profenofos	43.0% E. C.	1,000×
Methidathion	40.0% E. C.	800×
Cyanophenphos	25.0% E. C.	1,000×
Methamidophos	50.0% E. C.	1,200×
Prothiophos	50.0% E. C.	1,500×
Sannate**	45.0% E. C.	1,000×
Cypermethrin	5.0% E. C.	1,500×
Decamethrin	2.8% E. C.	1,000×
Fenvalerate	20.0% E. C.	4,000×
Flucythrinate	31.6% E. C.	8,500×
Permethrin	10.0% E. C.	3,000×
Cartap	50.0% S. P.	1,000×
Thiocyclam hydrogen oxalate	90.0% S. P.	2,000×
Carbofuran	40.6% F. P.	1,200×
Methomyl	90.0% W. P.	3,000×

\*not registered for DBM, tested here as a check insecticide

\*\*mixture of 30% phenthoate+15% dimethoate=45% a.i. of insecticides

## Results

1. **The actions of different insecticides:** the action of insecticide against DBM was measured by two major criteria i.e., the initial as well as the residual activity. Usually, the initial activity depended mostly on direct contact of insecticide, while the residual activity was the combined efforts through contact and feeding of treated leaves. The insecticidal activity patterns were determined on the native susceptible IL-strain DBM to minimize the possible distortion from resistance. For 18 organophosphorus insecticides, 5 synthetic pyrethroids, 2 carbamates and 2 tertiary amines, the test results of initial effects are reported in Table 2 and the residual effects are presented in Table 3 and Figure 1 to 5.

**Table 2.** The initial effect of 27 insecticides against both susceptible and resistant diamond-back moth larvae\*.

Insecticides	Mortality (%)		Insecticides	Mortality (%)	
	S**	R**		S**	R**
<u>Organophosphorus compounds</u>			<u>Synthetic pyrethroids</u>		
Pirimiphos-methyl	96.8	85.7	Cypermethrin	100.0	32.8
Diethquinalphion	72.7	48.3	Decamethrin	100.0	41.4
Mephosfolan	88.5	94.3	Fenvalerate	94.0	59.7
Dichlorvos	60.6	55.0	Flucythrinate	100.0	57.4
Diazinon	64.6	28.1	Permethrin	100.0	65.5
Naled	47.3	53.7	<u>Others</u>		
Phenthoate	100.0	83.6	Thiocyclam hydrogen oxalate	100.0	100.0
Mevinphos	100.0	100.0	Cartap	100.0	100.0
Pyridaphenthion	55.7	26.4	Carbofuran	77.7	85.0
Acephate	23.3	20.8	Methomyl	6.9	15.3
Parathion	97.3	88.9			
Salithion	68.3	40.0			
Profenofos	100.0	77.4			
Methidathion	100.0	98.3			
Cyanophenphos	91.1	100.0			
Methamidophos	89.8	62.5			
Prothiophos	100.0	100.0			
Sannate	95.0	77.1			

\*insecticide sprays at the registered concentrations

\*\*S: susceptible I-lan strain ; R: resistant Lu-chu strain

**Table 3.** Effective periods for different levels of residual action of 27 insecticides against both susceptible and resistant diamondback moth larvae.

Insecticides	Days for different levels of mortality					
	S**			R**		
	75%	50%	25%	75%	50%	25%
<u>Organophosphorus compounds</u>						
Pirimiphos-methyl	1.4	3.8	6.2	—	1.0	3.1
Diethquinalphion	—	1.1	6.6	—	—	2.4
Mephosfolan	6.1	17.4	28.7	—	21.2	43.3
Dichlorvos	—	—	—	—	—	—
Diazinon	—	—	1.5	—	—	—
Naled	—	—	—	—	—	—
Phenthoate	1.1	2.6	4.2	1.1	1.7	2.4
Mevinphos	—	—	2.7	—	—	—
Pyridaphenthion	—	—	1.2	—	—	—
Acephate	—	—	4.7	—	—	—
Parathion	—	—	—	—	—	—
Salithion	—	—	1.9	—	—	0.7
Sannate*	0.8	4.5	8.2	—	—	2.7
Profenofos	2.0	3.8	5.5	—	0.7	2.6
Methidathion	—	1.7	3.8	—	1.4	3.0
Cyanophenphos	6.3	11.9	17.5	3.9	6.4	8.9
Methamidophos	2.6	8.3	13.9	0.7	4.3	7.8
Prothiophos	4.2	8.3	12.5	4.1	6.3	8.5
<u>Synthetic pyrethroids</u>						
Cypermethrin	—	—	—	—	—	—
Decamethrin	—	—	5.1	—	—	2.9
Fenvalerate	—	—	—	—	—	—
Flucythrinate	—	—	0.9	—	—	2.5
Permethrin	—	2.2	6.0	—	—	—
<u>Others</u>						
Thiocyclam hydrogen oxalate	0.8	4.7	8.7	—	—	2.3
Cartap	1.2	7.9	14.6	—	3.8	8.5
Carbofuran	—	—	6.0	—	0.5	5.4
Methomyl	—	—	—	—	—	—

\*Sannate : 30% phenthoate+15% dimethoate

\*\*S : susceptible I-lan strain ; R : resistant Lu-chu strain

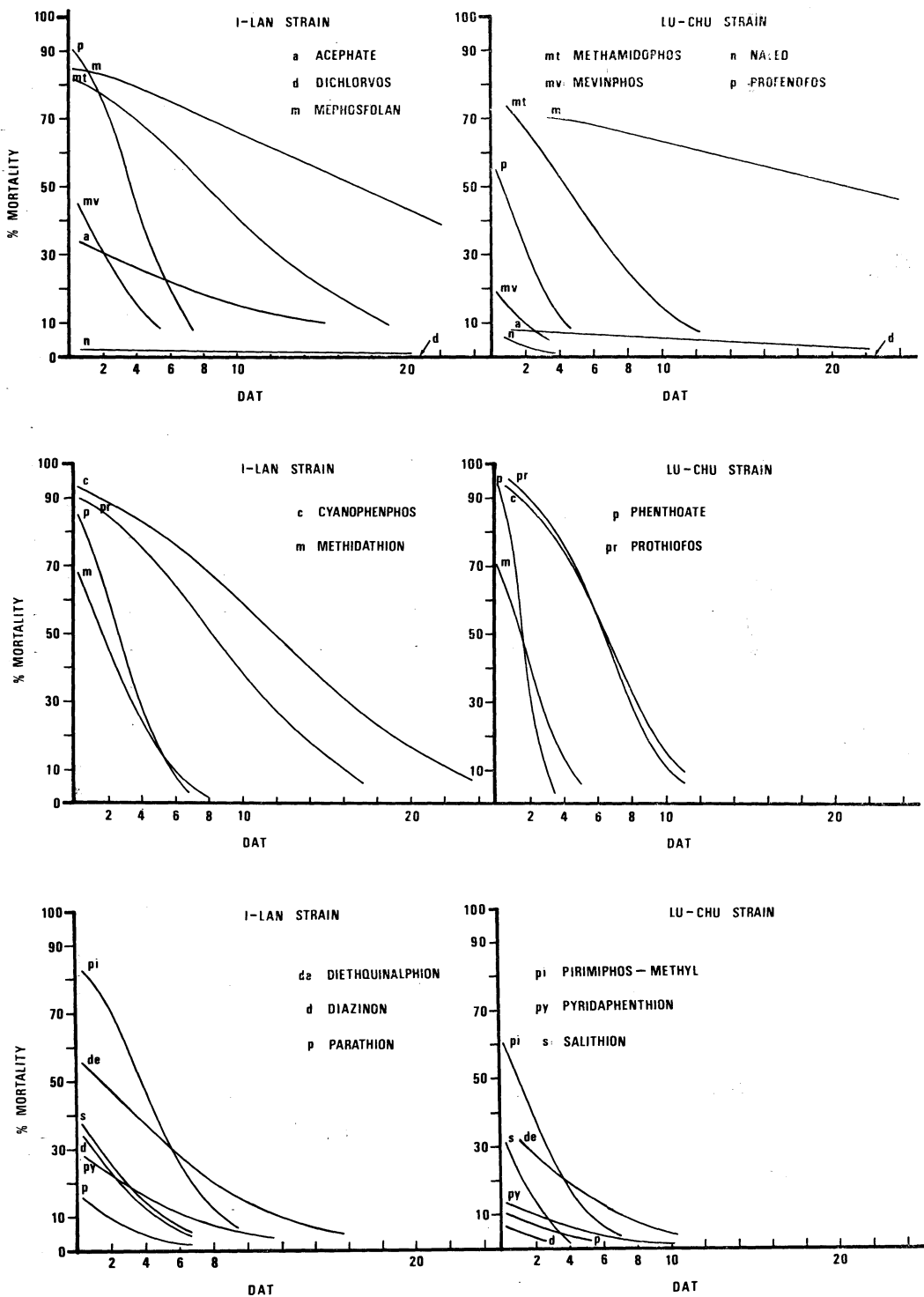


Figure 1~3 The residual activity patterns of organophosphorus insecticides on the susceptible (left) and resistant (right) diamondback moth larvae.

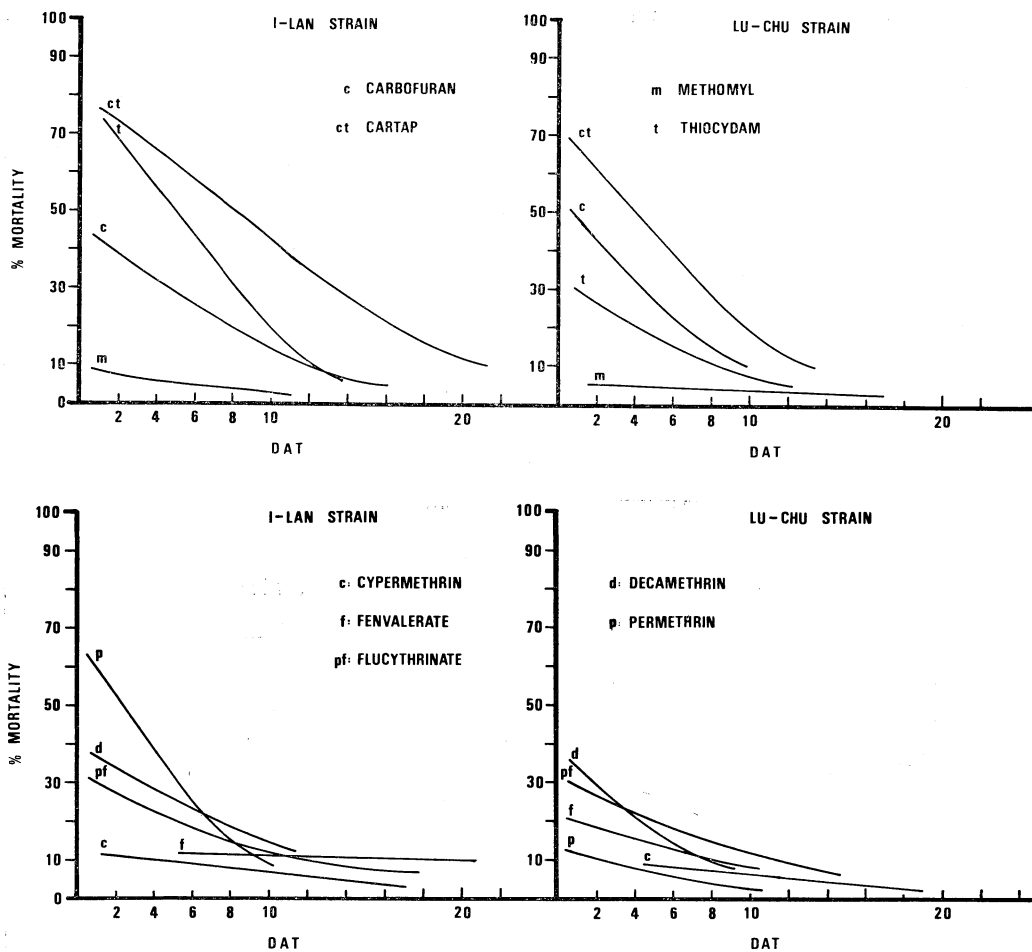


Figure 4~5 The residual activity patterns of carbamates, tertiary amines and synthetic pyrethroids on susceptible (left) and resistant (right) diamondback moth larvae.

For the initial effects, almost 100% mortality were recorded for pirimiphos-methyl, phenthoate, mevinphos, parathion, profenofos, methidathion and prothiophos; mephosfolan, cyanophenphos and methamidophos resulted in more than 85% mortality in DBM. Other organophosphorus compounds in this test did not provide good control on DBM. Synthetic pyrethroids and tertiary amines were excellent in their initial actions. Carbofuran provided 80% control, while methomyl showed almost no control at all at the recommended concentration.

In the test of residual effects, we have compared the activity patterns of insecticides according to their chemical nature (Figure 1 to 5). The organophosphorus insecticides are the largest chemical group tested and they demonstrated various action patterns. Mephosfolan, cyanophenphos, methamidophos and prothiophos were much persistent than others; dichlorvos and naled rapidly lost their control effects on DBM following the spray; other nine organophosphorus insecticides possessed moderate residual activities. Two tertiary amines and carbofuran had similar residual activity except the

strengths were different. Methomyl again showed less effective in its residual activity. All the synthetic pyrethroids tested emerged with the same pattern i.e., a good initial activity but followed with very poor residual effect.

The effectiveness of all tested insecticides decreased at different rates follow the time course, and their cross profiles at three strength levels i.e., 75%, 50% and 25% control efficacies are indicated in Table 3 based on number of days.

**2. The impact of resistance on the insecticide action pattern:** the LC-strain with multiple resistance was treated along with the IL-strain to examine the resistance impacts. The test results are included in the right columns of Table 2, 3 and part (b) of Figure 1 to 5. The resistance clearly reduced the effectiveness of insecticide in both initial and residual activities. In the test of LC-strain, synthetic pyrethroids were the most troubled insecticides and several organophosphorus insecticides were also suffered from strong resistance impacts. In general, once the insecticide efficacy was affected by resistance, the activity profile in Figure 1 to 5 was lowered and shortened.

**3. Validity of the test for detecting the resistance in field strains of DBM:** three groups of resistant DBM i.e., a laboratory-select resistant strain with known resistance origin; DBM sampled from areas with limited insecticide usage; and samples from insecticides intensively used areas, were tested against 13 insecticides and the results are compared in Table 4. For the prothiophos-resistant strain, a successful breeding of desired resistance from the susceptible IL-strain, not only insensitive to

**Table 4.** The initial effect of 13 insecticides against resistant diamondback moth larvae (percentage mortality).

Insecticides	Prothio- phos-R. strain	Matsu strains		Taiwan strains			
		MC	FH	BC	SY	TY	PT
Cartap	(%) 100	(%) 100	(%) 100	(%) 100	(%) 100	(%) 100	(%) 100
Thiocyclam hydrogen oxalate	100	100	100	100	100	100	100
Pirimiphos-methyl	46.2	92.0	82.0	93.1	83.3	89.3	78.3
Diethquinalphion	38.1	40.0	43.8	40.0	43.5	66.7	31.8
Mephosfolan	11.5	—	—	10.7	33.3	66.7	23.1
Phenthoate	100	96.0	90.0	100	90.0	76.9	72.7
Mevinphos	66.7	100	100	92.0	91.3	92.3	88.5
Profenofos	89.7	45.8	43.3	50.0	66.7	82.6	60.0
Methidathion	85.7	96.1	90.0	75.8	75.0	91.7	74.2
Methamidophos	33.3	70.0	69.0	0.0	8.3	4.2	8.0
Prothiophos	20.7	92.9	82.1	69.0	41.2	60.9	52.0
Decamethrin	53.8	—	—	14.3	12.0	42.3	8.0
Permethrin	83.3	84.0	100	26.7	70.8	52.0	63.3



prothiophos, also it became less sensitive to other insecticides due to the possible cross resistance except thiocyclam hydrogen oxalate, phenthoate, and cartap. The greenhouse testing method pinpointed the prothiophos-insensitivity in the known resistant strain served well as a positive check in demonstrating that the method is suitable for selecting effective insecticides to control DBM with unknown resistance origins.

The test results of field-collected DBMs have provided more evidences in supporting the desirability of the testing method. All four DBM populations from northwestern Taiwan still subjected to the control of cartap, thiocyclam hydrogen oxalate, mevinphos, methidathion and phenthoate, while other insecticides had lost their effectiveness due to the resistance. Permethrin recorded 25-70% mortalities when decamethrin could only result in 8-42% mortalities, which indicated the appearance of a strong synthetic pyrethroid resistance and matched exactly with our previous results<sup>(1,3)</sup>. Two DBM samples from Matsu isles exhibited lower insecticide resistance than that of DBMs from Taiwan. Two Matsu strains were more susceptible to most insecticides except diethquinalphion, profenofos and methamidophos. The insensitivity to profenofos may due to the cross resistance since profenofos had never been used there. Also, Matsu area has just begun introducing synthetic pyrethroids for agricultural pest control and one of the two DBM samples had showed early sign of resistance. Generally, the DBMs from Matsu isles still are susceptible to most insecticides as the area is not intensively cultivated.

### Discussion

During the investigation of insecticide resistance of DBM, we felt that the better understanding of insecticidal action from different aspects is needed in order to resolve the complicate intoxication and detoxication mechanisms<sup>(2)</sup>. The cuticle penetration and the oral ingestion are the two major entering ways for the toxicants. Insects with maximum exposure during the spray will subject to the strong initial action of the insecticide bath; on the other hand, those insects escaped from the initial contact of the spray would receive the toxicant through body contact in reducing degree and feeding of foliage with insecticide deposition. Since the initial and residual effects of insecticides are capable of killing insects, they should have received more attention in the resistance investigation. We hence adapted the testing method used by Hirano<sup>(6)</sup> to elucidate the actions of insecticides registered for DBM control in Taiwan.

As we had expected, the insecticides worked very differently. For example, at recommended dosages, synthetic pyrethroids worked perfectly as contact poisons, which had been demonstrated in their strong initial effects. Unfortunately, these dosages were not enough to cause stomach poisoning which contributed to their rather disappointing residual effects. Tertiary amines have high initial and long residual effects in killing the DBM. Carbofuran has been known for its effectiveness on the DBM<sup>(4)</sup> and we now confirm that it has the residual effect as well. Methomyl, on the other hand, is not effective either through contact or feeding<sup>(2)</sup>. Due to the diversity of molecular structures, different insecticidal action patterns were observed in the organophosphorus insecticides, which certainly offered greater choice for the DBM

control practice.

Another significant result can be concluded from the study is that this simple greenhouse testing method can successfully characterize the resistance profiles of a unknown DBM strain to insecticides. In our test results, the IL-strain was susceptible to almost all insecticides on the test list; the prothiophos-select strain showed a special tolerance to prothiophos accompanied with the cross resistance to some other organophosphorus insecticides; two strains from Matsu, an area with limited insecticide usage, were only resistant to few organophosphorus insecticides; and four strains from northwestern Taiwan exhibited high and multiple resistance to varieties of insecticides. The DBMs from different origins hence precisely reflected their level of resistance to insecticides and the greenhouse testing method can serve as a fast way to detect the effective insecticides for field DBM control.

If we applied the lock and key relation to the system of insecticides and resistance, the elucidated action patterns of different insecticides will be practical and helpful in probing the DBM resistance maze. The synthetic pyrethroid resistance is one good example to be discussed because the action patterns indicated that the selection pressure of synthetic pyrethroids obviously came from the contact rather than from the ingestion of toxicant. Therefore, we can make a rational presumption that the resistance mechanism is existed in the cuticle-epidermis system rather than the midgut-epithelium system, and the research effort should be concentrated on the penetration and detoxication of synthetic pyrethroids in the cuticle-epidermis of DBM. Similar clues can be applied on the investigation of other insecticides. Another hint obtained from the information is the comparison of action patterns of the same insecticide between susceptible and resistant DBM strains, for example, the cuticle-epidermis barrier should be investigated when the initial effect of insecticides reduced significantly; while the midgut-epithelium barrier should be analyzed if the residual effect was lowered and shortened in the resistant insects.

### References

1. Cheng, E. Y. 1981. Insecticide resistance study in *Plutella xylostella* (L.) II. A general survey (1980-81). J. Agric. Res. China 30 : 285-293.
2. Cheng, E. Y. 1985. The resistance, cross-resistance and chemical control of diamondback moth, *Plutella xylostella* (L.), in Taiwan. in Proceedings of the 1st Intern. Workshop on Diamondback Moth Management. AVRDC.
3. Cheng, E. Y., T. M. Chou and C. H. Kao. 1984. Insecticide resistance study in *Plutella xylostella* (L.) VI. An experimental analysis of organophosphorus and synthetic pyrethroid resistances. J. Agric. Res. China 34 : 96-104.
4. Chou, T. M. and E. Y. Cheng. 1983. Insecticide resistance study in *Plutella xylostella* (L.) III. The insecticide susceptibilities and resistance response of a native susceptible strain. J. Agric. Res. China 32 : 146-154.
5. Kung, K. S. 1984. Changes of economic importance of major insect pests of vegetables and prospects for their control in Taiwan. in Proceedings of the Symp. Insect Control of Vegetables in

Taiwan, Dept. of Agric. & Forestry, Taiwan Provincial Government. R. O. C.

6. Hirano, M. 1981. Controlling insect pests of chinese cabbage by fenvalerate and cyanophenphos, in "Chinese cabbage" Proceedings of the 1st Intern. Symp. AVRDC. p. 185-192.
7. Plant Protection Manual 1982 ed., Dept. Agric. & Forestry, Taiwan Provincial Government. R. O. C.
8. Personal communication with Ms Chen of Matsu Agric. Experi. Station. 1985.

## 小菜蛾抗藥性之研究

### VII. 防治藥劑之作用特性及受抗藥性之影響<sup>1</sup>

高靜華 鄭允 林端方<sup>2</sup>

#### 摘 要

本研究檢定目前登記用於防治小菜蛾，*Plutella xylostella* (L.)，殺蟲劑對本省感性品系宜蘭小菜蛾之初效及殘效。結果發現合成除蟲菊精類之作用均一致，在現有推廣濃度下，僅有良好的初效而殘效則不足，因此如蟲體未被藥液施及，僅靠乾涸於葉表面之藥膜使小菜蛾中毒的機會極低。18種供試的有機磷藥劑對小菜蛾之毒效因藥劑而不同，並且差別極顯著。初效以美文松、賽達松、佈飛松、滅大松及普硫松等最好；亞特松、施力松、達馬松及美福松則次之；巴拉松效果雖佳，但非蔬菜用藥。殘效方面以美福松最長，達馬松、施力松及普硫松次之，美文松、二氯松及乃力松均可謂無殘效。有機氮及氨基甲酸鹽類中，培丹及硫賜安之初效及殘效均佳，加保扶初效和殘效則屬中等。

抗藥性會使小菜蛾之初效反應下降，殘效反應縮短。測得之結果顯示，抗合成除蟲菊精劑之反應在感性及抗性品系小菜蛾頗一致，但抗有機磷之特性則隨藥劑之種類而有變化，一般而言，初效佳及致毒作用快者所受之影響較小，如美文松及賽達松。

同時亦探討利用本試驗之方法，即在網室以商品級農藥噴施推薦濃度之藥液於菜苗後接蟲測定毒效，檢定田間抗性小菜蛾之抗性特質，以選取防治該抗性品系之最佳藥劑。發現以室內經普硫松汰選之抗性小菜蛾供試時，可成功地測出該品系之（1）抗普硫松特性及（2）對數種其他有機磷之交互抗性。若以採自馬祖，對農藥抗性較單純之小菜蛾供試時，則可測出較本省西海岸各抗藥品系小菜蛾更為敏感之農藥感受性。再以（1）室內汰選之抗普硫松品系，（2）二個馬祖品系及（3）四個本省抗藥品系比較對13種常用殺蟲劑之感受性時，各品系之總體表現與其來源極為符合，故此一測試方法確實可作為鑑定未知抗性特質之田間小菜蛾品系之用。

1. 臺灣省農業試驗所 研究報告第 1265 號。本研究承中央加強農村建設補助計畫〔計畫編號74-農建-4.1-產植-28 (05)，74-農建-4.1-產植-147(03)〕及國科會計畫 (NSC-74-0409-B055-02) 補助，簡淑貝、盧燕鈴協助工作，謹此深致謝忱。

2. 本所應用動物系助理、研究員及計畫助理。臺灣省 臺中縣 霧峰鄉。