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The Resistance and Cross Resistance of Diamondback Moth, *Plutella xylostella* to *Bacillus thuringiensis* in Taiwan[1]

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Abstract: The *Bacillus thuringiensis* (B.t.) resistance has been examined in diamondback moth (DBM, *Plutella xylostella*), and 4 strains collected from vegetable fields in north(1), central(2) and south(1) of Taiwan were compared to the laboratory-maintained susceptible strain on their sensitivities to six commercial B.t. formulations. Tests were conducted on three fixed dosages i.e., 4,000-, 32,000- and 128,000-fold dilution, from original formulations. In the susceptible strain, three designated dosages caused 96～100%, 58～97% and 41～81% mortalities in contrast to 16～100%, 0～65% and 0～31% mortalities in 4 field strains. The significant lose of B.t. efficacy in the field DBMs has confirmed the existence of B.t. resistance. Thuricide, with its longest marketing history in Taiwan, suffered the worst resistance of R.R.=118.3. The estimated resistance ratios of field DBM to *B.t.* subsp.*kurstaki* are 55.4～118.3. For the single CryIA(c) toxin product, MVP, the resistance ratio is 34.9. Mild cross resistances of *B.t.* subsp. *kurstaki* to *B.t.* subsp. *aizawai* were detected, and the resistance ratios are 9.9～22.0.

By testing the laboratory-reared field DBM colonies on the same 6 designated B.t. formulations, the resistance ratios were 1.3～7.0, and the results indicated their B.t. sensitivities were similar to those of the susceptible strain. Hence the B.t. resistance in DBM will decline when the environment is free from the B.t. selection pressure. Field DBMs collected from different regions showed various insensitivities to all 6 B.t. insecticides, and demonstrated that DBM has responded to the long-term usage of B.t. products in Taiwan's intensively cultured vegetable fields.

Key words: *Plutella xylostella, Bacillus thuringiensis,* Subsp. *aizawai,* Subsp. *kurstaki,* Resistance, Cross resistance

INTRODUCTION

It is hard to define the susceptibility of an organism to its natural pathogens, since all epidemic factors have to be included for consideration. The biological variations of hosts, environmental components for inoculation, and the subsequent disease development, etc., are the possible reasons to restrain researchers from defining the resistance of a pest to its

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microbial pathogens. Although *Bacillus thuringiensis* (B.t.) kills its insect hosts mainly by toxins, the complexity of toxins and combined action of spores still obscured the definition of B.t. resistance. Hence, the B.t. resistance in the diamondback moth (DBM, *Plutella xylostella*) can not be demonstrated earlier until described by Tabashnik *et al.*⁽⁵⁾ on a specific crop in a clearly defined culturing environment and a well-documented B.t. application history. B.t. has been widely used in Taiwan for more than 30 years, and mostly served as a supplemental control agent for other chemical insecticides for example, organophosphates and carbamates. On the other hand, there has not a native B.t. susceptible DBM strain been maintained in Taiwan, which hampered the investigation of B.t. resistance in DBM because the study should best base on a native susceptible strain to distinguish the induced resistance from the natural tolerance. Investigation into the B.t. resistance in DBM was started from establishing a native B.t.-susceptible DBM strain, and the IL-strain that has been maintained in Taiwan Agricultural Research Institute (TARI) for more than 130 generations was compared to the Abbott Laboratories' susceptible DBM (AL-strain) in a parallel study.

MATERIALS AND METHODS

Insecticides

Six commercial B.t. formulations were used in this study. They were mostly developed and marketed recently except Thuricide. B.t. formulations were diluted with distilled water containing 333 ppm Triton X-100 to 4,000-, 32,000- and 128,000-fold for bioassay. The B.t. formulations tested were:

1. Javelin, 6.4% WG (*B.t.* subsp. ***kurstaki***, 32000IU/mg, Sandoz)
2. Thuricide HP, 3% SP (*B.t.* subsp. ***kurstaki***, 16000IU/mg, Sandoz)
3. Dipel 2X, 6.4% WP (*B.t.* subsp. ***kurstaki***, 32000IU/mg, Abbott Lab.)
4. Xentari, 10.3% WDG (*B.t.* subsp. *aizawai*, Abbott Lab.)
5. MVP, 10% AF (CryIA(C), Mycogen Corp.)
6. Turex, 3.8% WP (B.t. *aizawai*, transconjugated from *B.t.* subsp. ***kurstaki***, and *B.t.* supsp. *aizawai*, Ciba Geigy)

Commercialized chemical insecticides were used for testing the susceptibilities of IL- and AL-strain of DBM, and the formulations used were:

1. Fenvaletate, 20% EC
2. Permethrin, 10% EC
3. Cypermethrin, 5% EC
4. Mevinphos, 25.3% EC
5. Profenofos, 43% EC
6. Quinalphos, 25% EC
7. Methidathion, 40% EC
8. Methamidophos, 50% EC
9. Pirimiphos-methyl, 26.3% EC
10. Phenthoate, 50% EC
11. Carbofuran, 40.64% FP
12. Methomyl, 90% WP

Insect materials:

1. Susceptible strains:
   IL-strain (native of Taiwan, Pesticide Res. Lab., TARI)
   AL-strain (Abbott laboratories, USA)
2. Field resistant strains: collected in June-August, 1993.

FR1: collected from San-chung in northern Taiwan.
FR2: collected from Hsi-hu in central Taiwan.
FR3: collected from Hsi-lo in central Taiwan.
FR4: collected from Lu-chuin in southern Taiwan.

3. Laboratory maintained field colonies: field strains resistant to chemical insecticides that have been reared in laboratory free from insecticide selection since 1989.

L1 colony
L2 colony
L3 colony

Spray tower method:

Sensitivities of the susceptible IL-and AL-strain DBM to chemical insecticides were compared by using the Potter Spray Tower method. Commercial insecticides were diluted with distilled water into serial dilutions within the range of LC_{10} and LC_{90}, and 1 ml of test solution was sprayed on both sides of the leaf disc (ca. 7×7 cm^2). Each dosage was tested on total of 45—60 late 3rd instar larvae in three replicates(1). Mortalities were recorded at 24 hr after treatment and the results were analyzed in probit(2).

Bioassay method :

Leaf dipping method was used for testing the susceptibilities of DBM to B.t. Leaf discs with a diameter of 3.5 cm were cut from cabbage leaves which had been washed and air-dried. Twenty-four leaf discs for 4 replicates were prepared for each dosage and dipped for 5 seconds in B.t. solutions containing 333 ppm Triton X-100 and then left to air-dry. For the control test, cabbage leaf discs were dipped in distilled water with 333 ppm Triton X-100 only and then were air-dried on wire racks.

Four treated leaf discs and 15 late 3rd instar DBM larvae were put in a petri dish as one replicate in the beginning, and at 48 hr interval, the other two treated leaf discs been kept at 5°C were added for further observation. Total of 60 DBM larvae in 4 replicates were subjected to all treatments, and the mortalities were assessed at 72 hr after the start of treatment. Analyses were made by correcting with the control first, and then analyzed by probit(2). If the DBM mortality in the control lot was over 8%, the test was abandoned and repeated.

RESULTS

To select a proper susceptible strain for the study, both chemical and B.t. sensitivities of IL-strain and AL-strain were examined. Although IL-strain is highly sensitive when compared to field collected DBMs(1), it is more resistant than the AL-strain with respect to chemical insecticides (Table 1).

The B.t. susceptibilities of IL-strain to 6 commercial formulations in three dilutions were compared to the AL-strain (Table 2). For MVP and Turex, slightly lower LC_{50}s were obtained for the IL-strain than the AL-strain, while similar LC_{50} values were obtained in Dipel and Xentari, and only Javelin and Thuricide concluded higher LC_{50} values in the IL-strain than the AL-strain. In analysis, all the LC_{50} differences between two strains in respect of any B.t. formulation were not significant at 95% fiducial limit (F.L.).

Table 1. Comparison of sensitivities of AL-strain and the native susceptible IL-strain to three major chemical insecticide groups

Insecticides	LC_{50}, mg/liter	
	IL-strain	AL-strain
Synthetic pyrethroids		
Fenvalerate	8.3	3.1
Permethrin	14.0	8.1
Cypermethrin	19.0	1.6
Organophosphates		
Mevinphos	73.0	10.5
Profenofos	100.0	44.4
Quinalphos	230.0	5.5
Methidathion	270.0	11.9
Methamidophos	560.0	57.9
Pirimiphos-methyl		17.9
Phenthoate	670.0	44.4
Carbamates		
Carbofuran	120.0	20.7
Methomyl	3,400.0	21.1

Table 2. Sensitivities of two susceptible DBM strains to six commercial B.t. products

B. t.	IL-strain		AL-strains	
products	LC_{50}^{z} (Slope)	95% F. L.	LC_{50}^{z} (Slope)	95% F. L.
Javelin	2.3(1.61)	0.4～ 4.3	0.4(1.16)	0～ 2.1
Thuricide	13.0(1.18)	6.7～20.4	4.9(2.18)	1.6～ 7.4
Dipel 2X	2.9(1.73)	0.2～ 5.6	2.9(1.04)	0.3～ 6.6
Xentari	15.1(1.30)	8.5～23.3	10.4(1.36)	5.5～15.9
MVP	6.2(1.73)	2.6～ 9.3	10.6(0.52)	0.7～26.9
Turex	2.7(1.10)	0.3～ 6.0	9.9(1.45)	5.4～14.9

[z] Concentration: mg/liter

Comparative study was conducted by testing the susceptibilities of four field-collected DBM strains to six B.t. products at 4,000-, 32,000- and 128,000-fold dilutions, and the results were present in Table 3. In respect of the effect of dosage on different strains, the lowest dosage at 128,000-fold dilution of 6 tested B.t. products caused 41～81% mortalities in the susceptible strain, while 0～9% mortalities were observed in FR1-strain, 7～31% in FR2-strain, 0～17% in FR3-strain, and 0～12% in FR4-strain. The medium dosage, or the 32,000-fold dilution, caused 58～97% mortalities in IL-strain, compared to 3～30% in FR1-strain, 23～65% in FR2-strain, 0～21% in FR3-strain, and 0～23% in FR4-strain. The highest dosage, the 4,000-fold dilution, caused 96～100% mortalities in the susceptible strain in contrast to only 29～97%, 44～94%, 20～100% and 16～75% mortalities in the FR1-, FR2-, FR3- and FR4-strains, respectively.

The DBMs collected in Taiwan's intensively cultured vegetable fields had demonstrated 55- to 118-fold resistance to 3 commercial B.t. products derived from *B.t.* subsp. *kurstaki,* and the strongest resistance is for Thuricide with the longest marketed history in Taiwan (Table 4). Same field DBMs showed a medium degree of resistance to the single toxin product, MVP, while two newer B.t. products of *B.t.* subsp. *aizawai,* Xentari and Turex, also suffered 10- to 20-fold resistance, or 10—40% of the resistance for *B.t.* subsp. *kurstaki.*

TARI has maintained 3 colonies of field DBM in laboratory for more than 50 generations without any B.t. selection. These strains were also tested against the same 6 B.t. formulations at the same discriminating dosages (Table 3), and the resistance ratios ranged from 1.3 to 7.0 (Table 4), which were not significantly different from the susceptible DBM. Instead, all their 95% F.L. of LC_{50} were distinct from those of freshly collected field DBMs.

Table 3. Comparing the susceptibilities of the susceptible, laboratory-reared, and field strains to 6 B.t. products at 3 disriminating dosages

B.t. products	Dilutions[z]	Mortality (%)								
		Susceptible		Lab.-reared			Field			
		IL	AL	L1	L2	L3	FR1	FR2	FR3	FR4
Javelin	4000X	100	100	100	100	87	47	86	46	75
	32000X	97	99	94	64	87	17	44	10	15
	128000X	81	94	80	52	49	3	28	0	6
Thuricide	4000X	96	100	100	100	88	35	44	20	16
	32000X	60	97	86	76	26	10	27	8	0
	128000X	45	67	40	22	9	8	13	0	0
Dipel 2X	4000X	100	98	100	96	80	54	69	58	60
	32000X	97	85	75	53	41	15	25	17	13
	128000X	77	68	63	39	18	0	10	17	12
Xentari	4000X	97	98	100	98	91	68	88	59	27
	32000X	58	71	83	49	33	22	23	16	23
	128000X	41	46	51	43	5	9	7	6	0
MVP	4000X	100	73	100	100	77	29	94	55	36
	32000X	89	69	58	91	11	3	65	0	7
	128000X	58	42	44	57	9	2	31	0	6
Turex	4000X	100	100	100	100	98	97	86	100	60
	32000X	84	70	73	71	30	30	43	21	22
	128000X	73	49	24	52	15	7	19	3	5

[z] Equivalent dosages:
4000X = 250.0mg/liter
32000X = 31.3mg/liter
128000X = 7.8mg/liter

Table 4. The sensitivities and resistance ratios (R. R.) of the L- and FR-strains compared to IL-strain

B.t. porducts	Lab-strains(3)			FR-strains(4)		
	LC_{50}^{z}(Slope)	95%F. L	R.R.^{y}	LC_{50}^{z}(Slope)	95%F. L	R.R.^{y}
Javelin	4.2(0.99)	1.6～ 7.3	1.8	132.3(1.15)	93.5～ 207.3	57.5
Thuricide	21.1(1.66)	16.4～26.7	1.6	1,538.0(0.71)	531.7～17,812.4	118.3
Dipel 2X	16.6(1.09)	10.9～23.2	5.7	160.8(1.10)	110.4～ 267.9	55.4
Xentari	19.6(1.42)	12.6～28.6	1.3	150.1(1.23)	107.2～ 232.1	9.9
MVP	19.3(1.16)	11.1～30.0	3.1	216.4(0.96)	136.7～ 430.5	34.9
Turex	18.9(1.71)	13.1～26.3	7.0	59.6(1.66)	47.0～ 77.0	22.0

[z] Concentration: ㎎/liter.

[y] Resistance ratios (R. R). were calculated in respect of IL-strain.

DISCUSSION

Tabashnik(5) had judged from the mode of action study of B.T. that the resistance should result only from B.t. selection rather than from the cross resistance of other chemical insecticides. This point of view had been demonstrated and validated since both AL- and IL-strains have the same sensitivities to B.t., while their LC_{50}s to various chemical insecticides are different. The IL-strain was therefore chosen for the subsequent comparisons because it is a native strain of Taiwan, and genetically is more comparable to those field collected DBMs than the AL-strain.

Among different B.t. products, Thuricide, with its long history of usage, was not effective even in the highest testing concentration. Better performance at the highest concentration was observed for the newer B.t. products e.g., Javelin, Dipel 2X, Xentari and Turex, while MVP was effective only in the FR2-strain. The results also demonstrated that the B.t. resistance is strain-dependent e.g., the DBM collected from Hsi-hu (FR3-strain) was more susceptible to B.t. than the DBMs from other regions. This might be caused by the local variation of B.t. used as hypothesized by Tabashnik(5). The laboratory test results showed the average effectiveness of products in field DBM followed the order of Turex, Javelin, Xentari, Dipel 2X, MVP and Thuricide.

Since all 4 field strains were collected before any *B.t.* subsp. *aizawai* product was marketed, the possibility of a mild cross resistance from *B.t.* subsp. *kurstaki* to *B.t.* subsp. *aizawai* is obvious. The cross resistance between different isolates of *B.t.* subsp. *kurstaki* in DBM had been documented by Tabashnik *et al.*(5) and Shelton *et al.*(4), and also by McGaughey and Johnson(3) in *Plodia interpunctella*. Mild cross resistance of *B.t.* subsp. *kurstaki* to *B.t.* subsp. *aizawai* in DBM had been detected by Shelton *et al.*(4) and Tabashnik *et al.*(6), but the Taiwan field DBM showed stronger cross resistance between these two B.t . subspecies.

Due to the discovery of resistance and cross resistance of B.t., serious consideration should be given on the selection of different B.t. formulations for DBM control in respect of resistance management. Whether *B.t.* subsp. *kurstaki* and *B.t.* subsp. *aizawai* can be alternated should also be tested and discussed since different subspecies still have overlapped portions in toxin compositions. In cautious, we suggest the B.t. should be alternated with insecticides with chemical nature, or with a mode of action deviated from B.t., for example, the newly registered abamectin.

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小菜蛾對蘇力菌抗藥性及交互抗性[1]

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摘要

由三重、溪湖、西螺及路竹採得之小菜蛾以六種商品化之蘇力菌配方（Thuricide、Dipel 2X、Javelin、MVP、Xentari 及 Turex）測試其感度，並與室內之感性品系進行比較，發現所測試的三個稀釋濃度（4,000倍、32,000倍及128,000倍）下，感性品系之死亡率分別為為96～100％、58～97％及41～81％。而田間品系在相同的三個濃度下，死亡率則為16～100％、0～65％及0～31％，顯示蘇力菌對田間小菜蛾之防治效率已經降低，而證實蘇力菌之抗藥性確實已經發生，尤其以在本省使用歷史最長之 Thuricide 之抗性最高，其抗性比為118.3。經估計，所採得之田間小菜蛾對三種 *kurstaki* 亞種之蘇力菌商品，Javelin、Dipel 2X 及 Thuricide，之抗性比為55.4～118.3，對蘇力菌單一毒素之產品 MVP 抗性比為34.9。試驗中並檢測出 *B.t.* subsp. *kurstaki* 造成兩種 *B.t. subsp. aizawai* 產品 Xentari 及 Turex 之交互抗性，其抗性比為9.9～22.0。以採自相同地區，在室內飼養四年且未受蘇力菌汰選之田間品系測試，對蘇力菌之感度僅微次於感性品系，抗性比在1.3～7.0之間，與田間新採集之小菜蛾對蘇力菌感度有顯著之差異，顯示歷經一段無汰選時期後，小菜蛾對蘇力菌之敏感度會逐漸恢復。本研究確認本省小菜蛾已普遍對兩個蘇力菌亞種之商品均產生抗藥性，且其原因應來自田間長期使用蘇力菌所致。

關鍵詞：小菜蛾、蘇力菌、抗藥性、交互抗性、kurstaki 品系、aizawai 品系。

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[1] 臺灣省農業試驗所 研究報告第 1752 號。本研究承農委會經費補助〔（計畫編號：82科技－2.2－糧－11(7)及(8)、83－科技－2.4－糧－36(28)及(29)〕，洪秀芬、盧燕鈴及簡淑貝協助工作。

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